CAVE AND CLIFF SWALLOWS AS INDICATORS OF EXPOSURE AND EFFECTS OF ENVIRONMENTAL CONTAMINANTS ON BIRDS FROM THE RIO GRANDE, TEXAS

A Thesis

by

DANIEL MUSQUIZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2003

Major Subject: Wildlife and Fisheries Sciences

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Approved as to style and content by:

Miguel Mora (Co-Chair of Committee)	John Bickham (Co-Chair of Committee)
Duncan S. MacKenzie (Member)	Robert Brown (Head of Department)

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ABSTRACT

Cave and Cliff Swallows as Indicators of Exposure and Effects of Environmental Contaminants on Birds from the Rio Grande, Texas (August 2003)

Daniel Musquiz, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Miguel A. Mora
Dr. John W. Bickham

Cave (*Petrochelidon fulva*) and cliff swallows (*Petrochelidon pyrrhonota*) were collected along the Rio Grande and evaluated as potential indicators of environmental contamination. The Rio Grande receives toxic substances from agricultural, industrial, municipal, and non-point sources; consequently, high levels of contaminants have been detected in birds, mammals, fishes and sediments. Swallows were obtained from 8 sites between Brownsville and El Paso, as well as from a reference site in Burleson County, 320 miles north of the nearest site of the Rio Grande. Blood samples were analyzed by flow cytometry, a technique that allows the detection of DNA damage in blood and other tissues. Plasma samples were analyzed for thyroid hormones using a radioimmunoassay technique. Organochlorines and trace metal analysis was limited to a few samples. DDE and PCB levels were below levels known to cause reduced hatching, embryo mortality, and deformities, Hg, Pb, and As were below detection, and Se, Ni and Cr concentrations were lower than levels known to cause harm in birds. Neither species showed sex-related differences in chromosome damage. Cave swallows from the Del Rio area had the highest levels of DNA variation, which may be indicative of DNA damage, possibly from PAHs exposure. Previous studies indicate that sediment samples

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from tributaries near Del Rio have high levels of chromium compared to other sites along the Rio Grande. A significant increase in DNA variation between sampling years was detected in cave swallows from Llano Grande Lake. Wildlife samples collected from Llano Grande Lake have recorded high levels of DDE and PCBs; in addition, this urban/agricultural contaminant sink appears to be affected by PAH exposure. T_3 levels were below the detection limit of the radioimmunoassay. There were no gender related differences in T_4 levels in cave swallows. Cave swallows sampled from Laredo had significantly higher T_4 levels than those from birds at other sites during 1999. It was not possible to determine thyroid hormone disruption in plasma samples. Thyroid hormone and flow cytometry data were useful in establishing baseline data. Areas of concern based on genotoxic data include Llano Grande Lake, Del Rio, and El Paso.

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DEDICATION

To my parents, Roberto and Victoria, my sister, Rosalinda, and my brother, Jose Luis. It was their prayers, love, support, and encouragement that carried me through these years.

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I would like to thank the personnel from the United States Geological Survey, Biological Resources Division, specifically, Catherine Eyster for always keeping me informed and up to date with deadlines and standard procedures. I thank Mike Atkinson and Eladio Rivera for helping me collect samples out in the field. I would also like to thank Don Clark and Deborah Cowman for their encouragement during all those hours spent in the lab. I thank Donell Frank for introducing me to the field of wildlife toxicology while I was an undergraduate. Also, thanks to Jody Sandel for her guidance and encouragement while she completed her thesis. I also thank Cole Matson for his expertise in running the flow cytometer, as well as Carol Swartz for helping me with flow cytometry lab procedures, and Robert Trujillo for the assistance he provided while conducting flow cytometry experiments. I am also thankful to Kevin Leiner and Tom Gash for providing their knowledge and expertise on thyroid radioimmunoassays and associated software.

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Last, but not least, are the folks who kept me going though all these years. My brother's generosity and his confidence in my work provided the fuel that kept me going when times were tough. My uncle Roger and his late wife Lupe, are like second parents to me; I thank them for their hospitality while I traveled to and from Texas A&M. My sister Rosalinda and her words of encouragement always kept me going as well. And most importantly, my parents, for whom I am most grateful for everything they have done in helping me get to where I am today.

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INTRODUCTION

The Rio Grande originates in the San Juan Mountains of Southern Colorado (Texas Natural Resources Conservation Commission, TNRCC 1996a). In Texas, the river forms the international boundary with Mexico from El Paso to Brownsville, as it empties into the Gulf of Mexico (TNRCC 1996b). The Rio Grande is a vital resource for the populous areas of El Paso/ Ciudad Juarez, Eagle Pass/ Piedras Negras, Laredo/ Nuevo Laredo, McAllen/ Reynosa, and Brownsville/ Matamoros. Consequently, the Rio Grande is heavily impacted by industrial, municipal, and agricultural sources throughout the Texas-Mexican border.

In Texas, the Rio Grande meanders through various mountains, deserts, spring systems, wetlands and coastal estuaries (TNRCC 1996b). These distinct habitats support diverse wildlife communities, which are dependent on the health of the river and its tributaries. Sensitive wildlife such as the ocelot (*Felis pardalis*) and jaguarondi (*Felis yagouarundi*), have suffered population declines (TNRCC 1996b). Within the lower South Texas Plains and Gulf Prairies and Marshes, the United States Fish and Wildlife Service once considered 145 vertebrates as species that required immediate protection (Jahrsdoefer and Leslie 1988). In addition, 86 vertebrate species in the Lower Rio Grande Valley are considered endangered, threatened, or have been placed on a watch-list, by various state and federal agencies (Jahrsdoefer and Leslie 1988). Habitat destruction, as a result of agricultural practices and urbanization, accounts for most of

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wildlife population declines (TNRCC 1996b).

Davis (1995) suggests that the border's poor environmental status is a result of rapid population growth and industrialization. Recent expansion of the *maquiladoras* (mostly US-owned assembly plants) program and associated population relocation to the border area has contributed to increasing deterioration of water quality of the Rio Grande (Miyamoto et al. 1995). *Maquiladoras* have maintained high rates of growth since their introduction to Mexico; for instance, by early 1982, there were 600 plants employing 122,700 persons, and by September 1996, 2,490 plants employed 788,205 persons (INEGI 1996). Many of these facilities are known to use chemicals, such as organic solvents, acids, and metals in their production processes (Wainwright 1998). It is widely suspected and occasionally documented that *maquiladoras* illegally dump hazardous wastes in landfills, arroyos, and other sites in the vicinity of the Rio Grande (Texas Water Commission 1992; TNRCC 1996b;).

Agricultural activities also contribute to the poor water quality of the Rio Grande region (TNRCC 1996b). The Texas Water Commission (1992) estimates that agricultural use of surface water, on both sides of the Texas-Mexican border, is about 3.23 billion m^3. Both treated and untreated sewage effluents are often discharged into lateral irrigation canals or mixed with river water and used for surface irrigation in the Juarez Valley of Northern Mexico, near El Paso, TX (Assadian 1999). Water quality assessments in the El Paso region assert that high levels of nitrogen, phosphorus, and heavy metals are at levels of concern (IBWC 1994). Moreover, a survey of pesticide use in Texas between 1990 and 1993 revealed that a total of 13,268,017 kg of pesticides

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were used each year (Gianessi and Anderson 1995). Over 425,000 ha of cultivated land in the Lower Rio Grande Valley receive repeated applications of pesticides each year (Jahrsdoerfer and Leslie 1988). Pesticides currently approved for agricultural or domestic use in Texas (Table 1), have received more attention due to intentional use of synergistic chemicals used to increase the toxicity of less active compounds (Walker 1998).

Previous studies have reported exposure of municipal, industrial, and agricultural contaminants in fish and birds of the Rio Grande (Mora 1995; Mora 1997; Mora et al. 1997; Mora and Wainwright 1998; Mora et al. 2001; Wainwright et al. 2001). DDT (1,1,1-trichloro-2,2-bis [P-chlorophenylethane]), a persistent pesticide that was used since the 1940s and banned in the 1970s (Stickel et al. 1984), has been found in wildlife tissue samples mostly as DDE (2,2-bis[P-chlorophenyl]-1,1-dichloroethene) (Mora 1995). Mora and Wainwright (1997) noted that DDT and its metabolites have accounted for the majority of organochlorines reported in 52 aquatic and terrestrial birds of the Rio Grande. DDE levels were as high as 71 µg/g in gulls (*Larus atricilla*) collected from Llano Grande Lake in 1978 (White et al. 1983) and 46 µg/g in a white pelican (*Pelicanus erythrorhynchos*) carcass from the Pharr Settling Basin in 1986 (Gamble et al. 1988). Polychlorinated biphenyl (PCB) residues, a group of chemicals once used as coolants, insulating materials, and lubricants in electrical equipment (TNRCC 1996a) have also been found in wading birds and fish of the Lower Rio Grande Valley. Levels of PCBs, ranging from 0.53-9.6 µg/g, have been reported for fish samples (Texas Department of Health, TDH 1995; TNRCC 1994; Davis 1995). In 1993, a Lower Rio

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Table 1. Suggested insecticides for management of cotton insects in the LRGV (Norman and Sparks 2001).

Insecticide class	Insecticide
Carbamate	Carbaryl
	Methomyl
	Oxamyl
	Thiodicarb
Organophosphate	Chlorpyrifos
	Methyl Parathion
	Acephate
	Dicrotophos
	Dimethoate
	Oxydemeton-methyl
	Profenofos
	Azinphosmethyl
	Malathion
Synthetic pyrethroid	Cyfluthrin
	Cyhalothrin
	Cypermethrin
	Deltamethrin
	Esfenvalerate
	Bifenthrin
	Fenpropathrin
	Tralomethrin
	Zeta-cypermethrin
Cyclodiene	Endosulfan
	Propargite
Nitroguanidine	Imidacloprid
Ideno-oxadiazine	Idoxacarb
Insect growth regulator	Methoxyfenozide
	Deflubenzuron
	Tebufenozide

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Grande Monitoring Study sponsored by the EPA/Public Health Service, U.S. Department of Health and Human Services, and the State of Texas detected PCB (Arochlor 1254) levels higher than 2 ppm, with the highest being 9.6 ppm (TNRCC 1994). As a result from this study, the TDH issued a fish consumption advisory in June 1993 for the Donna Reservoir and all irrigation canals in Hidalgo County, as well as, the North Floodway of the Arroyo Colorado (TNRCC 1994). Although DDT and PCBs are banned, there is concern about the persistent nature of these chemicals in the environment. These compounds are known to concentrate in fat tissues of organisms, and due to their lipophilic properties, bioaccumulate in animals at higher trophic levels (Froese et al. 1998).

Increased environmental chemical exposure worldwide has researchers concerned with the potential endocrine disrupting effects that man-made industrial chemicals and agricultural pesticides may have on wildlife (U.S. EPA 1998). Chemicals known to cause endocrine disruption are thought to mimic natural hormones, inhibit the action of hormones, or alter the normal regulatory function of immune, nervous, and endocrine systems (Crisp et al. 1998). Direct exposure to environmental toxicants, during prenatal and/or early postnatal life, whether persistent or short-lived, can have a deleterious and irreversible effect on a developing organism (Colborn et al. 1993; Kavlock et al. 1996). Thyroid hormones, for instance, are known to affect reproduction, growth, metabolism, temperature regulation, and behavior (Norris 1996), especially critical during avian development (Janz and Bellward 1997; Olson et al. 1999; Gould et al. 1999). Environmental agents, such as PCBs and dioxins, are known to mimic and

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alter thyroid hormone levels in wildlife (Crisp et al. 1998), and also have neurotoxic effects (Safe 1994). For instance, ortho-substituted PCBs are known to produce neurotoxic responses by decreasing dopamine levels in brain tissues and by altering cholinergic and muscarinic receptors in rodents and primates, respectively (Safe 1994). Moreover, PCBs and dioxins are known to compete for binding to the thyroid hormone receptor, thus acting as weak agonists and blocking the action of thyroid hormones (Porterfield 1994). Because of structural similarities, binding characteristics of PCBs and dioxins resemble those of thyroid hormones (Porterfield 1994).

Endocrine disruption is further supported by evidence that hormone levels fluctuate with the introduction of known chemicals in a dose-response fashion by either increasing or decreasing circulating hormone levels. For example, a study by Spear and Moon (1985) observed decreases in serum T_3 and T_4 concentrations in adult ring doves (*Steptopelia risoria*) injected intra peritoneally with 3,3',4,4'-tetrachlorobiphenyl. Janz and Bellward (1997) detected elevated plasma total T_4 concentration in TCDD-exposed great blue herons; however, no effects were found on total T_3 levels or on plasma T_3 to T_4 ratio.

Current knowledge of environmental contaminants in biota of the Rio Grande suggests that birds from this region may be exposed to contaminants, such as, polycyclic aromatic hyrdrocarbons (PAHs), trace metals, and certain organochlorines (Mora and Wainwright 1997), among others, some of which are known to cause DNA damage. Clastogens, agents that cause chromosomal aberrations (Hoffman, 1996), exert their effects by promoting chromosome breakage or by chromosome rearrangements.

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Chemical interactions with DNA may lead to structural alterations in DNA molecules and may take the form of adducts, strand breakage, or chemically altered bases (Shugart et al. 1992). DNA lesions not successfully repaired may result in alterations that become fixed and eventually transmitted to daughter cells (Shugart et al. 1992). Chemical clastogens are also known to induce chromatid aberrations, which result from DNA synthesis on a damaged DNA template in the synthesis period of the cell cycle (Bender et al. 1998). DNA aberrations, if not corrected by DNA repairing mechanisms, may elevate the rate of introduction of new cytogenetic and allelic variants (Herbert and Luiker 1996), and consequently increase DNA variation. Birds that reside in different colonies along the Rio Grande may be exposed to localized genotoxic agents, and resulting genetic damage may ultimately be revealed by flow cytometry (FCM). Flow cytometry is an efficient and rapid technique that has been used for detecting genotoxic effects of environmental pollutants to wildlife populations (Otto and Oldidges 1980; McBee and Bickham 1988; Bickham 1990; Shugart et al. 1992; Custer et al. 1994; Bickham et al. 1998; Custer et al. 2000). Flow cytometry assays can detect changes that result from nuclear DNA strand breaks from tissues in which cellular or nuclear suspensions can be obtained (Bickham et al. 1998). Experiments have shown that mutagenic chemical exposure results in a broader range of variation in nuclear or chromosomal DNA content in a positive dose-response relationship (Shugart et al. 1992). For example, Otto and Oldiges (1980) measured the effects of cyclophosphamide, a known mutagen, on mice cells, and found that exposed cells had higher coefficients of variation (mean DNA content), than the control site. McBee and

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Bickham (1988) and McBee et al. (1987) also confirmed the usefulness of FCM as an indicator of DNA damage by reporting significant differences in cell DNA content between wild rodents from a petrochemical contaminated site and a control site. Custer et al. (2000) were able to show a direct correlation between elevated PAH concentrations in tissues and somatic chromosomal damage in blood of lesser scaups (*Aythya affinis*). A recent study suggests that TCDDs and specific mixtures of TCDD, 2,3,7,8-pentachlorodibenzofuran (PeCDF) and PCB 126 result in dose-dependent increases in the production of superoxide anion lipid peroxidation and DNA damage in hepatic and brain tissues in rats (Hassoun, et al. 2001). Although these effects have not been tested in wildlife with flow cytometry techniques, these findings suggest that certain organochlorines may potentially cause genetic insult in stressed individuals and warrants further investigation. Although it is unfeasible to screen all bird samples for organochlorines and trace metals, data from previous studies coupled with genetic biomarkers of exposure from this study provide a method for assessing the exposure and potential effects of contaminants on insectivorous birds, such as cliff and cave swallows.

Cave (*Petrochelidon fulva*) and cliff swallows (*Petrochelidon pyrrhonota*) were selected for this study due to their abundance, ubiquity, nest fidelity, and insect diet composition, which support their use as a good indicator species for monitoring uptake of environmental contaminants. Cave swallows are the North American swallows most closely related to cliff swallows (Brown and Brown 1995). Cliff swallow nest sites are typically chosen and established by March, when the first rounds of migrants arrive. Texas populations of cave swallows are known to move south during winter, although reports since the mid 1980s have indicated that cave swallows regularly overwinter in Texas (Lasley and Sexton 1991; West 1995). Cliff swallow are known to inhabit open canyons, foothills, and river valleys that offer a vertical cliff face with a horizontal overhang for nest attachment (Brown and Brown 1995); however with widespread development, colonies are now commonly found under large bridges. On the other hand, cave swallows inhabit open country areas of low elevation, and prefer suitable structures, such as, caves and culverts, where they usually attach their mud nests (Kirchman et al. 2000). Most cliff swallows forage within 1.5 km radius of the colony (Brown et al. 1992); however pre and postbreeding birds are known to feed in distances in the range of about 14 km (Brown and Brown 1996). These limited feeding ranges provide a means of monitoring contaminant uptake in localized environments during breeding activities.

A recent study on passerines (Klemens et al. 2000) found that insectivores had higher levels of organochlorine pesticides than non-insectivores, such as, granivores and frugivores. Higher organochlorine levels found in insectivorous birds might be attributed to the physical and chemical properties that pertain to these chemicals. For example, in natural waters, contaminant transfer among trophic levels is mainly controlled through factors, such as, pH, temperature, organic content, and contaminant solubility, which all control bioavailability. Benthic communities, because of their position amid the sediment layer and water column, are highly exposed and may rapidly absorb contaminants (Andres et al. 1998). Burrowing species may be exposed to contamination from two routes; for example: uptake of metals from the water through

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cutaneous and respiratory barriers, and absorption in the gut from ingested sediment (Andres et al. 1998). As insects undergo their early developmental stages, their potential to accumulate heavy metals and organohalogens peaks. Once these insects mature and emerge from their aquatic habitats, they become potential prey in higher trophic levels of the food chain.

Cave and cliff swallows parallel the use of tree swallows (*Tachycineta bicolor*) as bioindicators for studying the uptake of organohalogens and trace metals from insects that arise from aquatic habitats (Bishop et al. 1995; Froese et al. 1998; Harris and Elliot 2000). These studies found positive correlations between PCB concentrations in sediments and emergent insects, as well as, nestling tree swallows. Interspecific interactions observed by West (1995) indicate that cave swallows, cliff swallows, tree swallows and barn swallows (*Hirundo rustica*) are known to feed in the same flocks. Cliff swallow diets are mainly composed of homopterans, dipterans, hymenopterans, coleopterans, neuropterans, ephemeropterans, hemipterans, lepidopterans, orthopterans, and odonates (Brown and Brown 1995).

Continuing exposure of wildlife that inhabit the Rio Grande and surrounding areas, to environmental contaminants that exhibit potential endocrine disrupting and genotoxic properties prompted further investigation into the subject matter. Numerous studies have focused on the effects of contaminants on fish-eating birds of the Rio Grande (Mora 1996a; Mora 1996b; Wainwright et al. 2001); however, passerines are a group of birds that have been under-represented in ecotoxicological research despite recent declines in certain species (Ankley and Giesy 1998). Researchers suggest that

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organochlorine pesticide contamination may be a reason for these declines (Klemens et al. 2000). This investigation focuses on the potential effects of environmental contaminants on the insectivorous and migratory, cliff and cave swallow species. The collection of baseline data is an appropriate first step in evaluating the effects of pesticides on population dynamics of Neotropical migrants (Klemens et al. 2000). The purpose of this study was to assess the use of cave and cliff swallows as indicators of environmental contamination along the Rio Grande Basin by evaluating their thyroid hormone concentrations, DNA content (variation), contaminant load, and morphometric measurements.

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MATERIALS AND METHODS

Sites Locations

Study sites were located along the Rio Grande with one reference site 300 miles north of the Rio Grande as follows, Brownsville (25˚57'45''N, 97˚24'50''W), Llano Grande Lake (26˚07'15''N, 97˚57'66''W), Pharr/San Juan (26˚09'86''N, 98˚10'33''W), Mission (26˚11'40''N, 98˚19'79''W), Zapata (26˚50'98''N, 99˚15'92''N), Laredo (27˚24'67''N, 29˚28'83''W), Del Rio (29˚19'82''N, 100˚50'02''W), El Paso (31˚39'37''N, 106˚19'19''W, and 31˚35'73''N, 106˚17'57''W) and Somerville (25˚57'45''N, 25˚57'45''W) (Fig. 1). Carcasses and blood samples were obtained between June 9 and July 3 in 1999 and between May 17 and June 18 in 2000.

Sample Collection and Preparation

Eight nesting colonies were sampled along the Texas side of the Rio Grande in 1999 and five colonies in 2000. Most blood samples and specimens were collected between 7:30-10am to minimize the daily fluctuations in hormones and also because it was more convenient to work during those hours. Mist nets were strategically placed over entrances of culverts and sides of bridges to capture birds that were attempting to gain access or flying away from their nests. In a few cases, nestlings were collected directly from nests. Once captured, birds were placed in nylon bags for up to 15 minutes until blood collection. After blood collection, all birds were euthanized by cervical dislocation. Amount of blood collected, body weight and gender were documented for each bird. Blood samples were collected from the jugular vein using 25-gauge needles

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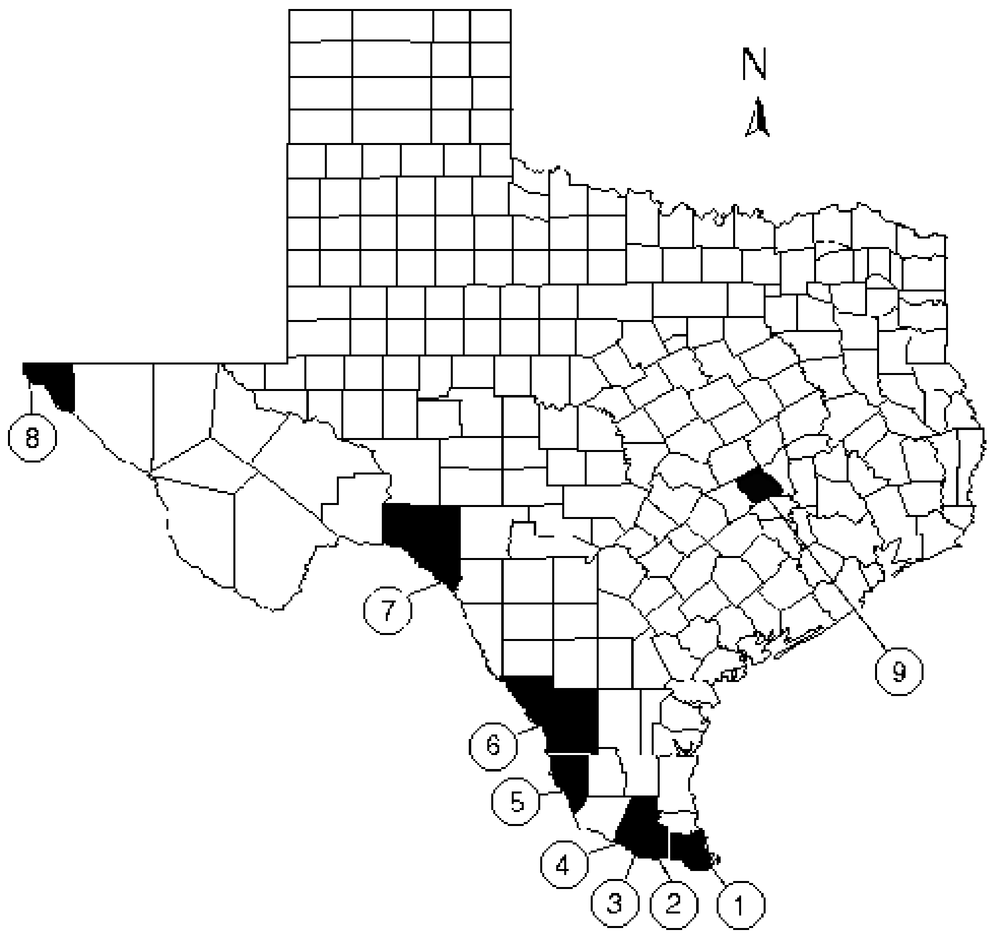

Figure 1. Collection sites for cave and cliff swallows during May and June 1999-2000.

Map Code	Location	Description
1	Brownsville	Brownsville Ship Channel, FM 511 & FM 48
2	Llano Grande Lake	Llano Grande Lake & FM 1015
3	Pharr-San Juan	Wastewater treatment plant & golf course, I Rd. & Gato Rd.
4	Mission	FM 1016 & Main Floodway
5	Falcon Lake	Northern branch of Falcon Lake, South of Zapata, TX
6	Laredo	San Idelfonso Creek, .6 km from Rio Grande
7	Del Rio	Zacatosa and Zocorro Creek, South of Del Rio on Hwy 277
8	El Paso	Wastewater treatment plant, Riverside Canal
9	Somerville	FM 1361, 1 km N Somerville, TX

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on 1ml tuberculin syringes and placed in 3 ml blood collection tubes containing 45 USP of sodium heparin. Each blood-filled tube was immediately placed on ice until all samples were collected. Approximately 2 hours after field collection, blood tubes were centrifuged for 15 minutes at approximately 1,500 rpm. The plasma was temporarily frozen in dry ice until permanently stored in an ultra cold freezer (-80˚C). Prior to centrifugation, 3-5 drops of whole blood were preserved in 500 µl of freezing media (Ham's F10 media with 18% fetal calf serum and 10% glycerin) and placed in cryogenic viles for flow cytometry analysis. Blood and media were thoroughly mixed and allowed to saturate; then stored in dry ice, and ultimately stored in an ultra cold freezer (-80˚C) until analysis.

Carcasses were transported to the laboratory for further analysis. In the lab, each carcass was reweighed with an Ohaus® microbalance to the nearest hundredths of a gram. Feathers surrounding the thorax and abdomen were removed. A bilateral incision was made from the cloaca, through the ribs, and up to the clavicle to expose internal organs. The spleen, liver, and gonads were removed and weighed with an Ohaus ® GT 410 analytical balance to the nearest thousandths gram. Sex for each bird was determined. When appropriate, condition and developmental stages of ovarian follicles were documented. Gonads in male birds were measured with vernier calipers. Spleen and gonads were placed in 2ml cryovials. Livers were wrapped in foil paper and stored at -80˚C for future analyses.

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Chemical Analysis

Due to the large amount of bird samples necessary for this research and the contaminant screening costs, only six samples were submitted for contaminant analysis. These birds, 2 from Llano Grande Lake, and 4 from El Paso, were analyzed at the Geochemical and Environmental Research Group (GERG), of Texas A&M University. The small sample size of birds analyzed for contaminants were not enough to derive conclusive results for thyroid hormones radioimmunoassays; however, to further support any contaminant issues, fairly recent publications that address contaminant levels of the Rio Grande were also evaluated.

Flow Cytometric Analysis

Flow cytometry is a technique in which DNA is stained with propidium iodide (PI) to promote fluorescence and detection within the flow cytometer. Propidium iodide stains both RNA and DNA; therefore, an RNAase is used to minimize chemical interferences. A visual output provided by the flow cytometer allows the user to discriminate between the periods of the cell cycle to be analyzed. This process, known as gating, allows the investigator to focus on the specific phases of the cell cycle, such as, the G1, S, and G2. DNA content of each cell in organisms is highly uniform (Rabinovitch 1994). Cells that have been stained with a dye that stochiometrically bindS to DNA produce a narrow distribution of fluorescence intensities (Rabinovitch 1994). Variability in dye binding to DNA, in practice, results in Gaussian (normally distributed) fluorescence distribution in G1 cells (Rabinovitch 1994). Greater variation in DNA content results in broader distribution peaks, and the term coefficient of variation (CV) is

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used to describe the width of the peak: CV = 100 x SD/mean of the peak (Rabinovitch 1994).

Flow cytometry was conducted with protocols developed previously (Bickham 1990, Bickham et al. 1998; Custer et al. 1994, 1997; Vindelov and Christensen 1994; Wickliffe and Bickham 1998). Briefly, whole blood samples were thawed to room temperature. Nuclear suspensions were obtained by mixing 50 µl sample blood, 50 µl citrate buffer and 450 µl trypsin solution. The mixture was homogenized by physical disruption with a teflon dounce 3-4 times, mixed by inverting the tube 3 times, and allowed to sit for ten minutes before adding 375 µl of trypsin inhibitor. After ten minutes, the mixture was filtered through a 30 µm nylon mesh into 6 ml, sterile, gamma-irradiated polypropylene tubes. Finally, 375 µl of propidium idodide stain solution were added to each tube, then covered with foil, and allowed to stain for ten minutes. *Gallus domesticus* blood cells were prepared and analyzed with the same procedures outlined above, to minimize mechanical error and ensure laboratory procedures (Wickliffe and Bickham 1998). Sample preparation and analysis sequences were randomly selected. A Coulter Elite flow cytometer was used to assay 10,000 cells/ sample, and estimate mean DNA content and half-peak coefficient of variation (HPCV) in DNA content. HPCV is calculated by taking the area under the distribution curve starting from the point that is half the height of the distribution curve.

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Thyroid Hormone Radioimmunoassays

For the RIA, a standard curve was created by serial dilutions of hormone with RIA buffer (15.46g barbital : 0.5g EDTA : 900ml ddH_20, pH to 8.6 : 0.1g thimerosal : 1.0g bovine gamma globulins) in duplicate at 50 µl. The standard curve ranged from 15.6 to 125 pg/tube. Plasma samples for T_4 and T_3 assays were prepared by diluting plasma with RIA buffer to 50 µl at 1:20 and 1:5, respectively. Each tube received 50 µl of rabbit anti- T_3 or T_4 (anti-serum), and additional 150 µl of radio-labeled T_3 and T_4 tracer (New England Nuclear) diluted to 25,000-30,000 cpm/tube with RIA buffer. All tubes were covered with foil, shaken vigorously for about one minute, and incubated at 37˚C for 1.5 hours and then overnight at 25˚C. To obtain a precipitate, 500 µl a solution composed of 5% polyethylene glycol (PEG) and a 1:20 second antibody (goat anti-rabbit gamma globulin) was added to every sample tube, and incubated at 4˚C for 1 hour. RIA tubes were then centrifuged at 3,200 rpm for 15 minutes with a Beckman J-6 centrifuge maintained at 4˚C. Each tube was inverted on absorbent paper for 20 minutes to decant the supernatant. Once drained, each tube was counted for 1 minute with a gamma counter (LKB-Wallac Riagamma 1274). These samples were assayed and analyzed by RIAMenu (developed by Paul Licht, University of California, Berkeley).

Validation of Radioimmunoassays for Cave Swallows

Validations for RIAs were conducted for cave swallows, and involved three experiments, including a range test, a parallelism test, and a recovery test. Intraassay and interassay variability were measured to further validate the RIA for cave swallows.

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For the range test, cave swallow plasma and plasma from wild and captive animals, were analyzed by the RIA (Leiner et al. 2000). The range test examines the range of concentrations of 3,5,3', 5'-tetraiodothyronine (L-thyroxine or T_4) and 3,5,3'-triiodothyronine (T_3) present in plasma from the wild populations along the Texas segment of the Rio Grande.

A recovery test is a useful method to measure the accuracy of a RIA for the particular species under investigation. Plasma samples may possibly contain compounds that interfere with accurate measurement of the hormone. Thus, recovery assays assist in accounting for the percent loss of an analyte and may detect interfering substances. For cave swallow pool 1 (CSP1) 150 μl of plasma were randomly selected and pooled from 3 samples collected from Pharr San Juan, 3 samples from El Paso and 2 samples from birds collected from Del Rio. For the second cave swallow pool (CSP2), 150 μl of plasma were randomly taken from 5 samples from Pharr San Juan, 2 samples from Del Rio, and 1 sample collected from El Paso. A control was created for each pool by taking 250 μl from each pool and adding 5 μl MeOH. A buffer control was created by adding 5 μl MeOH to 250 μl RIA buffer. For each cave swallow pool, one aliquot of 250 μl was spiked with 5 μl of a 10ng/ml dose of native T_4, while the second aliquot of 250 μl received 5 μl of methanol and served as a control. All control and supplemented samples were analyzed by RIA using the same procedures listed above to determine the percent of native hormone that could be recovered from the swallow plasma.

Parallelism tests are used to establish that hormones measured in swallow plasma by the RIA dilute similarly to native T_3 and T_4. While the presence of parallelism does

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not prove similarity of unknown and standard, lack of parallelism suggests dissimilarity in assay conditions between plasma and the assay buffer (Midgeley et al. 1969). For the parallelism test, CSP1 and CSP2 were used, in addition to plasma pools created from cave swallows sampled from El Paso (EPPL, 360 µl total, 120 µl each for 3 birds) and Del Rio (DRPL, 375µl total, 275 µl for 1 bird, 100 µl for a second bird) in 1999. CSP1, CSP2, EPPL, and DRPL were serially diluted with RIA buffer. Standard T_3 and T_4 were also serially diluted from 1.95 pg/tube to 125 pg/tube. All samples and standard dilutions were assayed with methods described above.

The plasma samples were evaluated to determine if variation in thyroid hormone levels were detected in cave and cliff swallow samples experiencing similar environmental conditions. Based on results from the range test, plasma samples for T_4 were analyzed at a 1:20 dilution and 50 µl (2.5 ng/ml) was measured by the RIA. The range test indicated T_3 was below detection for this RIA; T_3 was therefore not analyzed. The same procedures as described above were used for cave and cliff swallow plasma analysis. For all RIAs, plasma that was dark red in color was considered hemolyzed and removed from thyroid hormone analyses.

Statistical Analysis

Flow cytometry data from 1999 were log transformed to meet the assumptions of normality. A two-sample t-test was used to test for differences in thyroid hormone and FCM data between male and female birds from each individual site for both years. One-way analysis of variance (ANOVA) was used to measure differences between sites for each sampling session. When necessary, Tukey's pairwise comparison test was used to

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identify the location(s) that were significantly different. A two-sample t-test was used to compare year-to-year differences in thyroid hormone and FCM data. Statistical analyses were performed with Minitab for Windows Release 12.1. All results were considered significant at $P < 0.05$.

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RESULTS

Morphometric Measurements

Mean total body mass in cave swallows collected from Laredo in 1999 was significantly higher (Figure 2, $P < 0.05$) than those from Del Rio and Llano Grande, but not higher than those from Mission and Pharr-San Juan. The mean total body mass for all cave swallows collected in 1999 was 20.4g. Spleen weights in cave swallows from Del Rio were significantly higher than in swallows from Laredo and Mission (Figure 3, $P < 0.05$) but not in swallows from Llano Grande and Pharr-San Juan. Geometric mean spleen mass for these birds was 2.32 mg. Mean liver mass for the same group of birds was not significantly different among sites (Figure 4, $P < 0.05$). Mean liver mass for cave swallows sampled in 1999 was 0.69g. Gonad mass in male cave swallows did not differ statistically among sites (Figure 5), and averaged 34.6 mg. Likewise, gonad mass between female cave swallows was not statistically different among sites (Figure 6), and averaged 3.62 mg (geometric mean).

Mean total body mass in cliff swallows from El Paso was significantly higher than in those from Falcon Lake (Figure 7, $P < 0.05$), but not from Brownsville. Mean total body mass for all cliff swallows sampled in 1999 was 18.88 g. Spleen mass did not significantly differ among cliff swallows from El Paso, Falcon Lake and Brownsville (Figure 8), and averaged 3.67g (geometric mean). Liver weights in cliff swallows from Brownsville and El Paso were significantly higher (Figure 9, $P < 0.05$) than those in cliff

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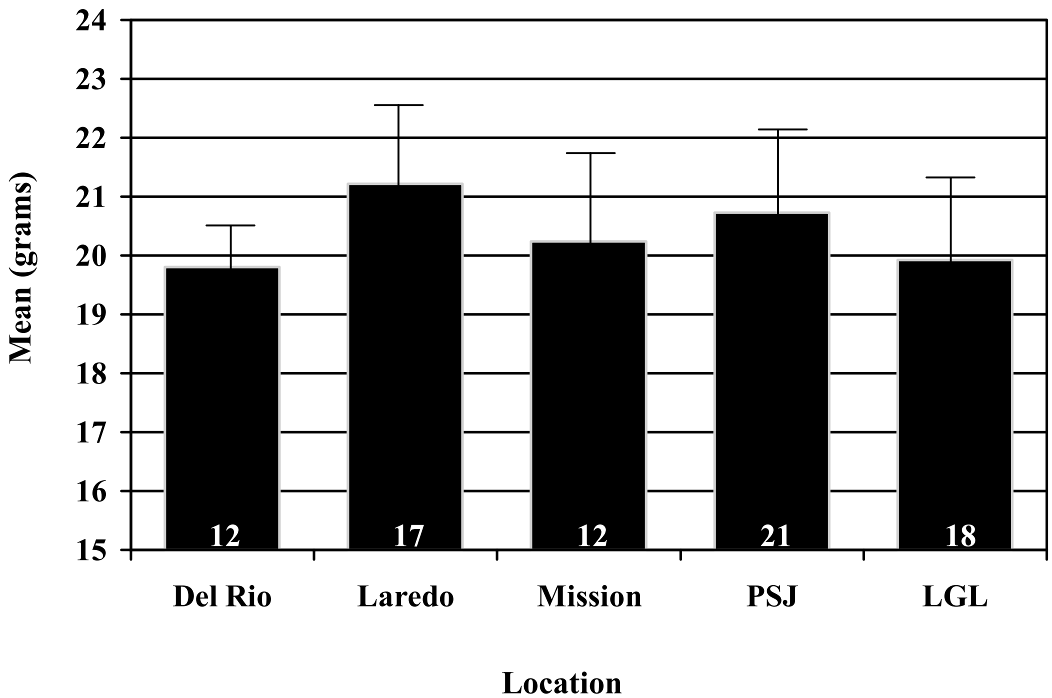

Figure 2. Total body mass from cave swallows (*Petrochelidon fulva*) collected from various sites along the Rio Grande during 1999. Bars represent standard deviations. Numbers in bars represent sample size.

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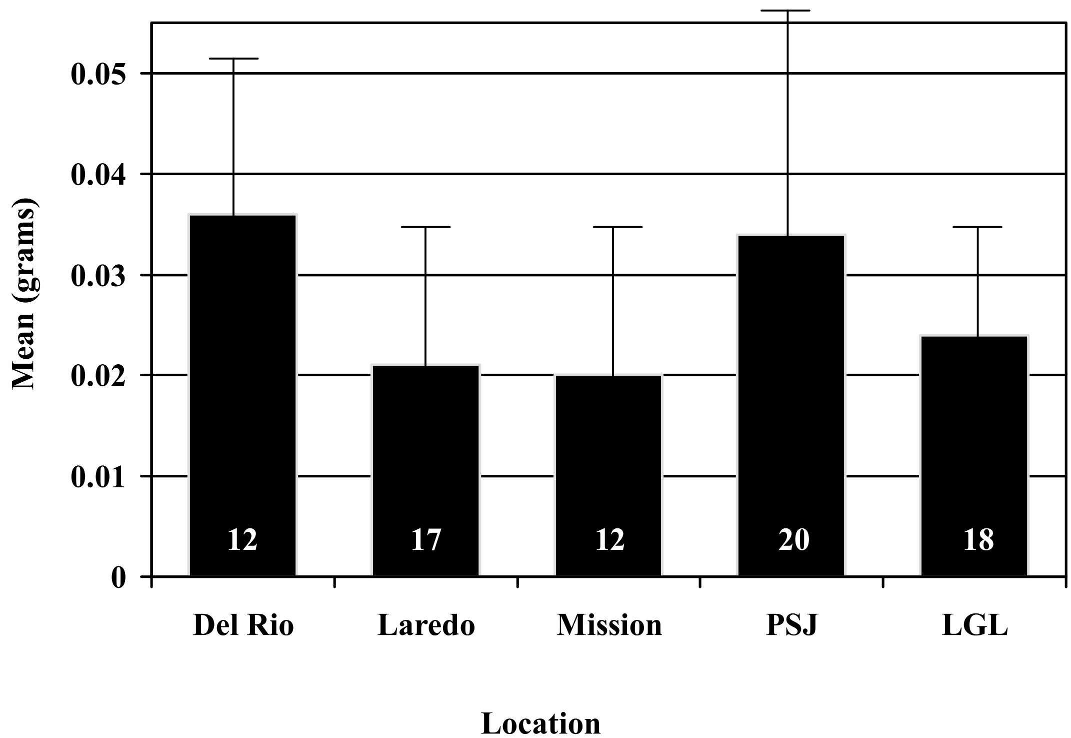

Figure 3. A comparison of spleen mass from cave swallows (*Petrochelidon fulva*) collected from various sites along the Texas segment of the Rio Grande during 1999. Bars represent standard deviations. Numbers in bars represent sample size.

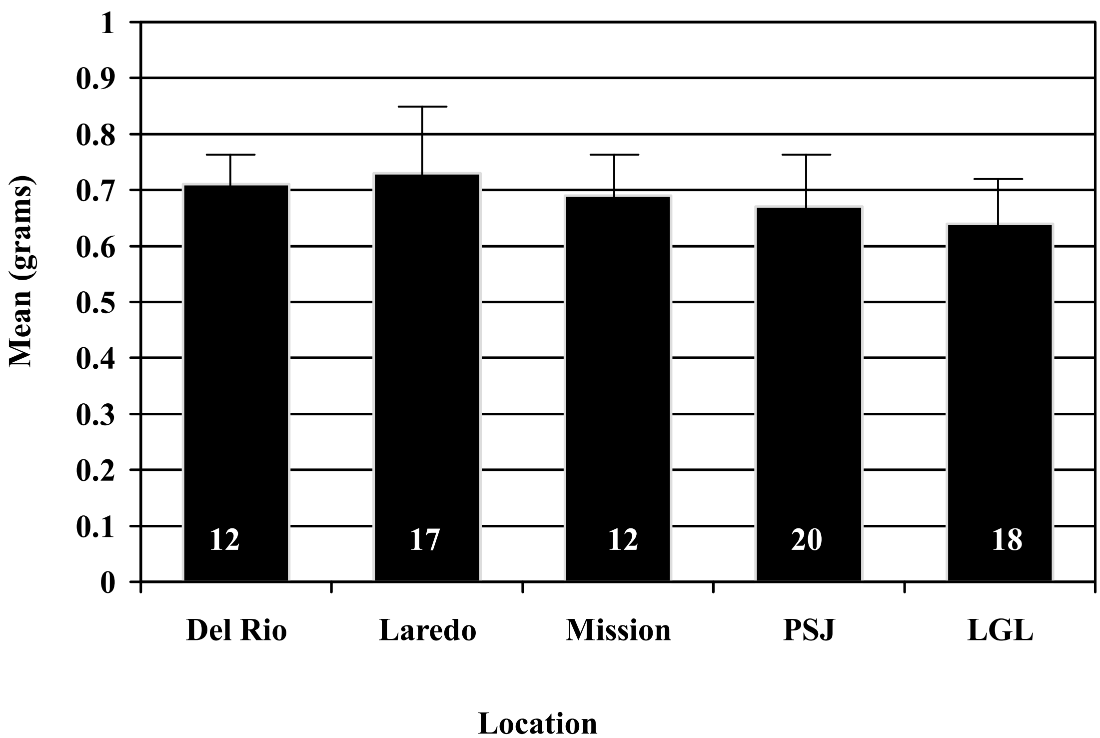

Figure 4. Comparison of liver mass from cave swallows (*Petrochelidon fulva*) collected from various sites along the Texas segment of the Rio Grande during 1999. Liver mass did not differ significantly between the sites. Bars represent standard deviations. Numbers in bars represent sample size.

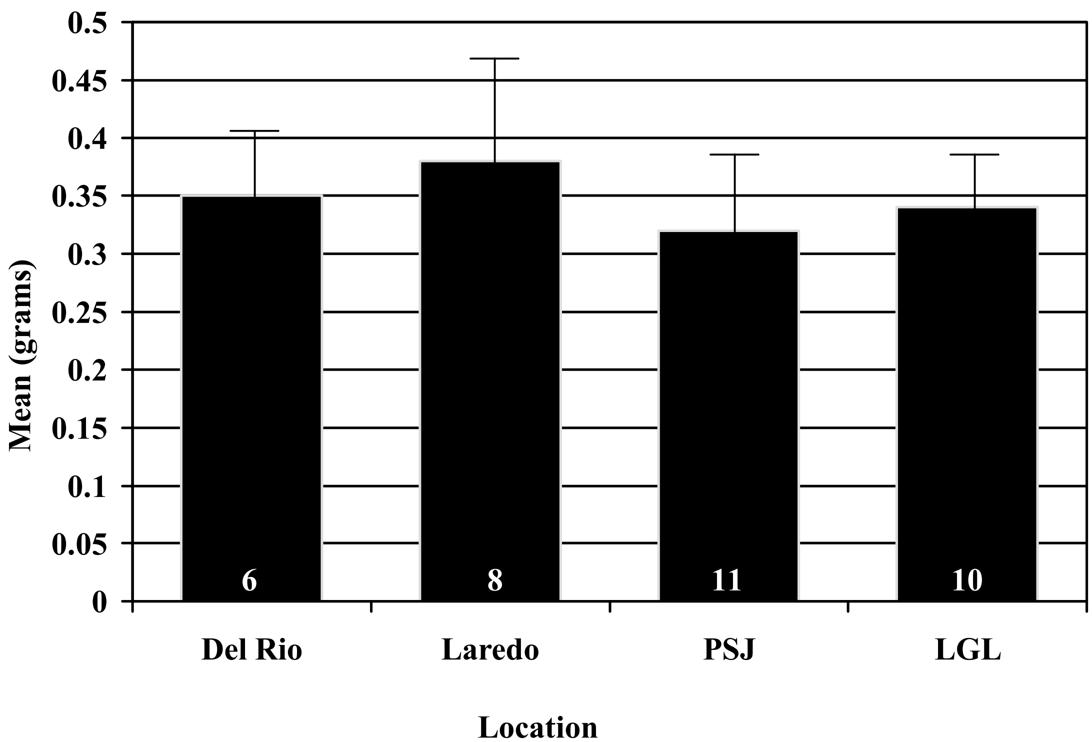

Figure 5. A comparison of gonad mass in male cave swallows (*Petrochelidon fulva*) collected from various sites along the Texas segment of the Rio Grande during 1999. Bars represent standard deviations. Numbers in bars represent sample size.

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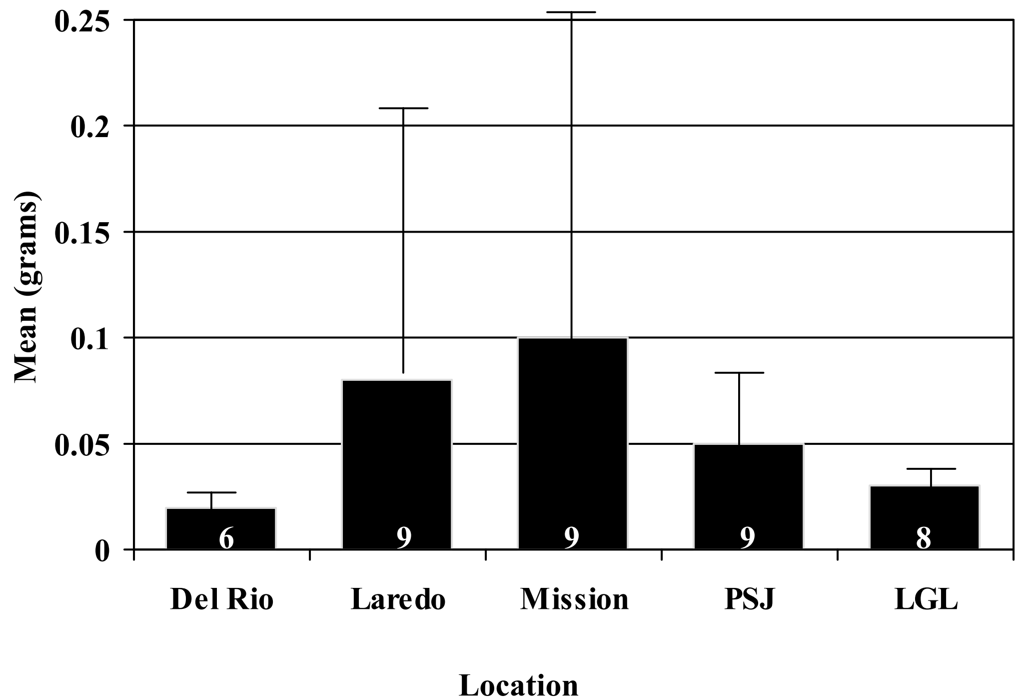

Figure 6. A comparison of gonad mass in female cave swallows (*Petrochelidon fulva*) collected from various sites along the Texas segment of the Rio Grande during 1999. Bars represent standard deviations. Numbers in bars represent sample size.

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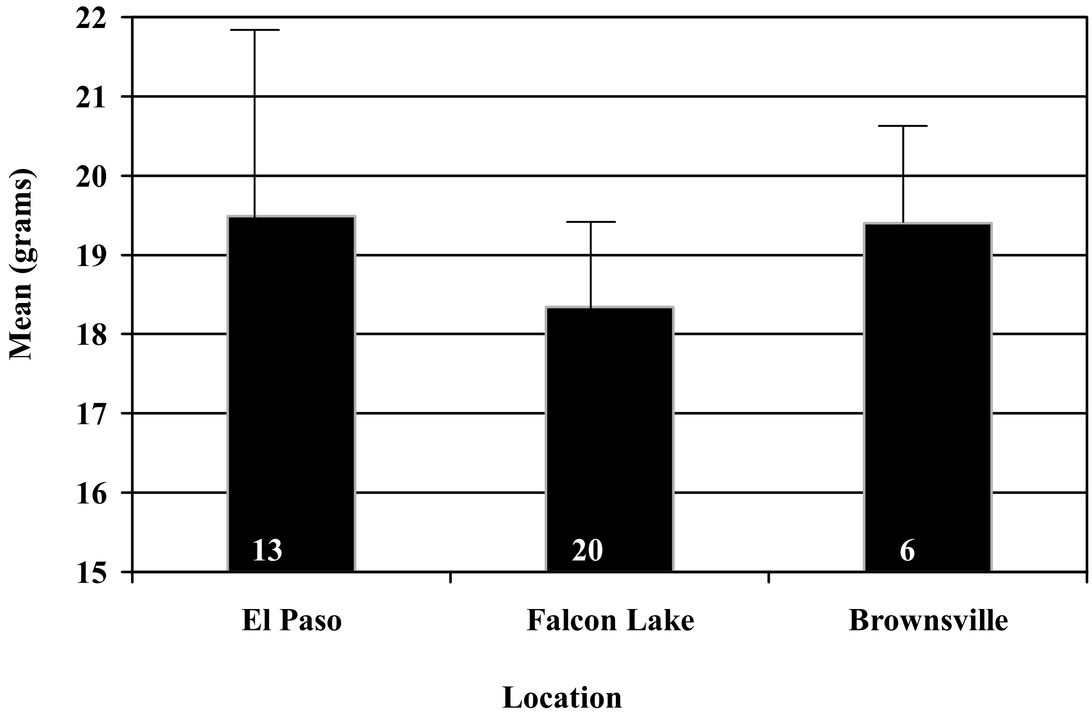

Figure 7. Total body mass from cliff swallows (*Petrochelidon pyrrhonota*) collected from various sites along the Rio Grande during 1999. Bars represent standard deviations. Numbers in bars represent sample size.

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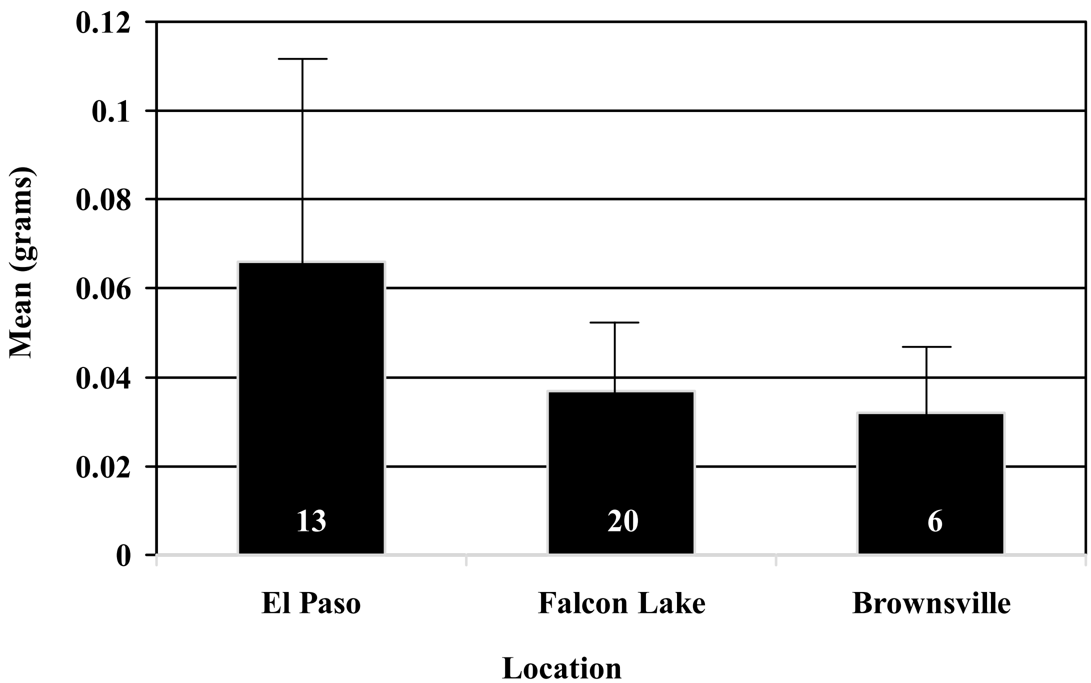

Figure 8. A comparison of spleen mass from cliff swallows (*Petrochelidon pyrrhonota*) collected from various sites along the Texas segment of the Rio Grande during 1999. Bars represent standard deviations. Numbers in bars represent sample size.

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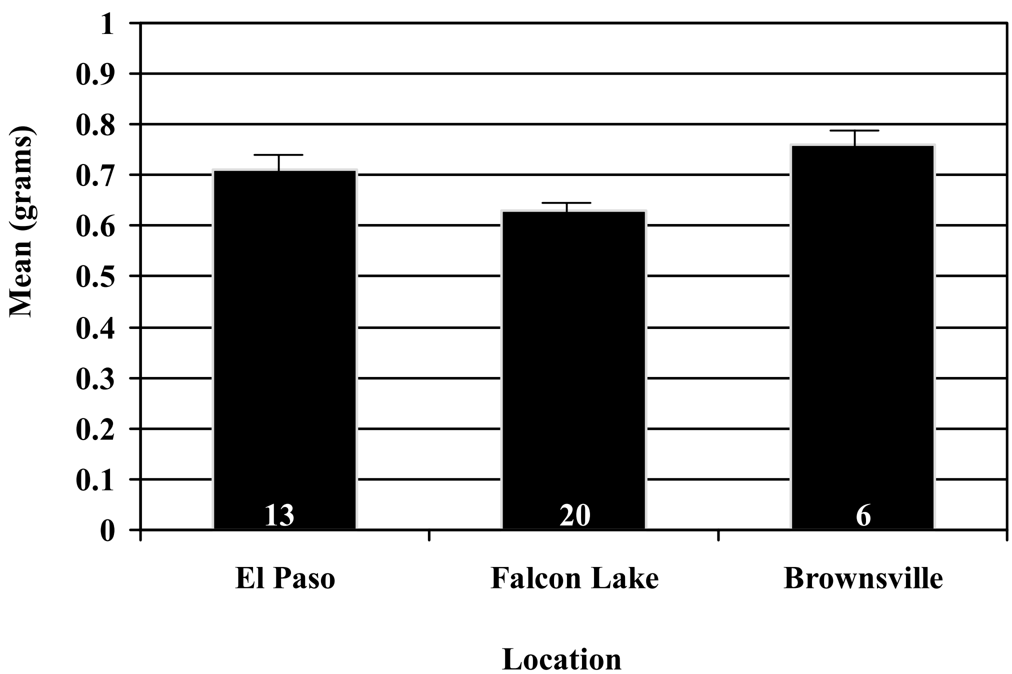

Figure 9. A comparison of liver mass from cliff swallows (*Petrochelidon pyrrhonota*) collected from various sites along the Texas segment of the Rio Grande during 1999. Bars represent standard deviations. Numbers in bars represent sample size.

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swallows from Falcon Lake. Cliff swallows sampled during 1999 had a mean liver mass of 0.67 g. Male gonad mass did not differ significantly between cliff swallows from El Paso and Falcon Lake (Figure 10). Not enough female cliff swallows were available to measure differences in gonad mass between sites.

Organochlorines

Due to the sample sizes, statistics were not performed on organochlorine contaminant concentrations. Furthermore, only birds from El Paso and Llano Grande Lake were analyzed for chemical residues. DDE was detected in all six samples selected for contaminant screening. The highest DDE levels (12,530 ng/g ww) and DDT levels (11.67 ng/g ww) were found in El Paso cliff swallows sampled in 1999. Polychlorinated biphenyl residues were detected in all cliff and cave swallow carcasses. The highest PCB concentration (630 ng/g ww) and lowest (288 ng/g ww) were detected in cave swallows from Llano Grande Lake. Of the hexachlorohexanes, the most prevalent was beta HCH. From the 1999 field samples, a cliff swallow from El Paso had the highest alpha HCH concentration (1.34 ng/g ww), beta HCH (35.76 ng/g ww) and gamma HCH (1.07 ng/g ww). For the chlordane-related compounds, heptachlor was not detected in any screened sample. Heptachlor expoxide, the metabolite of heptachlor, was detected in 5 out of 6 samples, and ranged from 0.89-2.46 ng/g ww. A cave swallow from Llano Grande Lake had the largest oxychlordane concentration (24.62 ng/g ww), two times greater than the next highest samples. No residues of alpha-chlordane were detected in tissue samples from Llano Grande Lake, however 2 out of 3 El Paso cliff swallows had low concentrations (1.63 ng/g ww and 0.33 ng/g ww). No residues of gamma-chlordane

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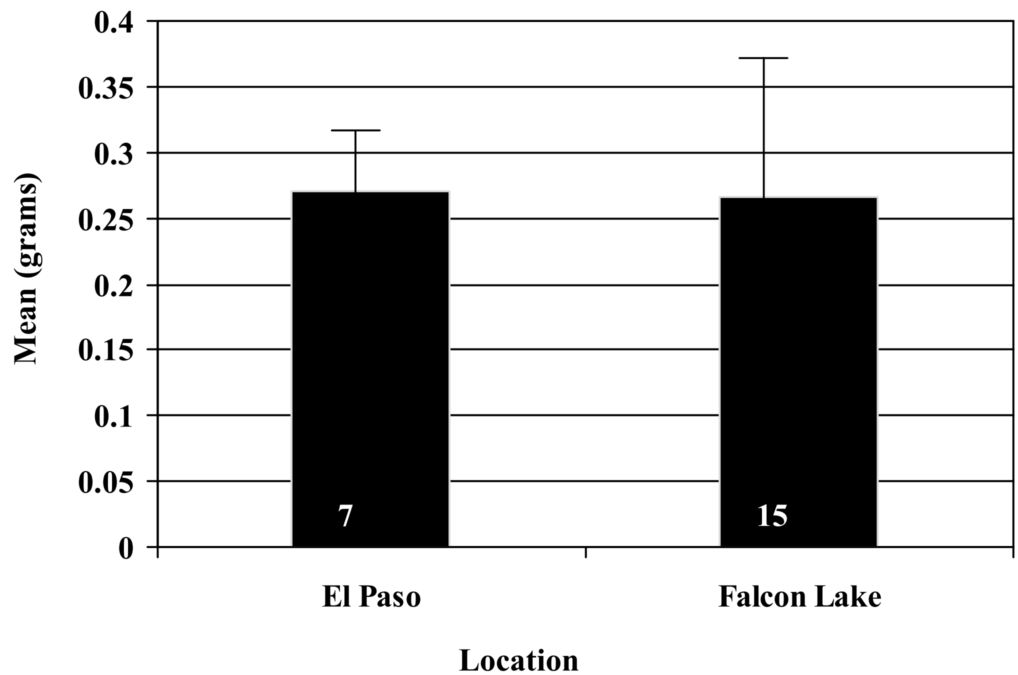

Figure 10. A comparison of gonad mass in male cliff swallows (*Petrochelidon pyrrhonota*) collected from El Paso and Falcon Lake in 1999. Bars represent standard deviations. Numbers in bars represent sample size.

were detected in any samples. Concentrations of Cis-nanochlor were below 1.80 ng/g ww in all samples. Four out of 5 samples had residues of trans-nanochlor, with the highest being from El Paso (7.56 ng/g ww). Aldrin was the only cyclodiene pesticide not detected in any of the six samples. Dieldrin was detected in both Llano Grande Lake samples (2.43 ng/g ww and 5.24 ng/g ww); but the highest dieldrin concentration was found in an El Paso (EP-5) cliff swallow sample (27.79 ng/g ww). Endrin was detected in one out of two Llano Grande Lake samples (1.85 ng/g ww) and in two out of four El Paso samples (0.39 ng/g ww, 0.51 ng/g ww).

Trace Metals

Hg, Pb, and As were below detection in all swallows. Se concentrations ranged from 0.37-0.79 ppm ww. Cd levels in cave swallows from Llano Grande Lake were below detection; however, Cd ranged from 0.05-0.11 ppm ww in cliff swallows taken from El Paso. Cr concentrations ranged from 0.22-0.41 ppm ww in all six samples. Ni was only detected in one sample, 0.50 ppm ww, from Llano Grande Lake. Al concentrations ranged from 7.57-23.1 ppm ww.

Flow Cytometry

No significant differences (Figure 11, $P > 0.05$, in all cases) in HPCV of DNA content were detected between male and female cave swallows from each location for both years. Gender differences were not statistically evaluated for cliff swallows, but sample sizes were small. Cave swallow samples obtained from Brownsville in 2000 were not included in the analysis due to a small sample size as well. Data for male and

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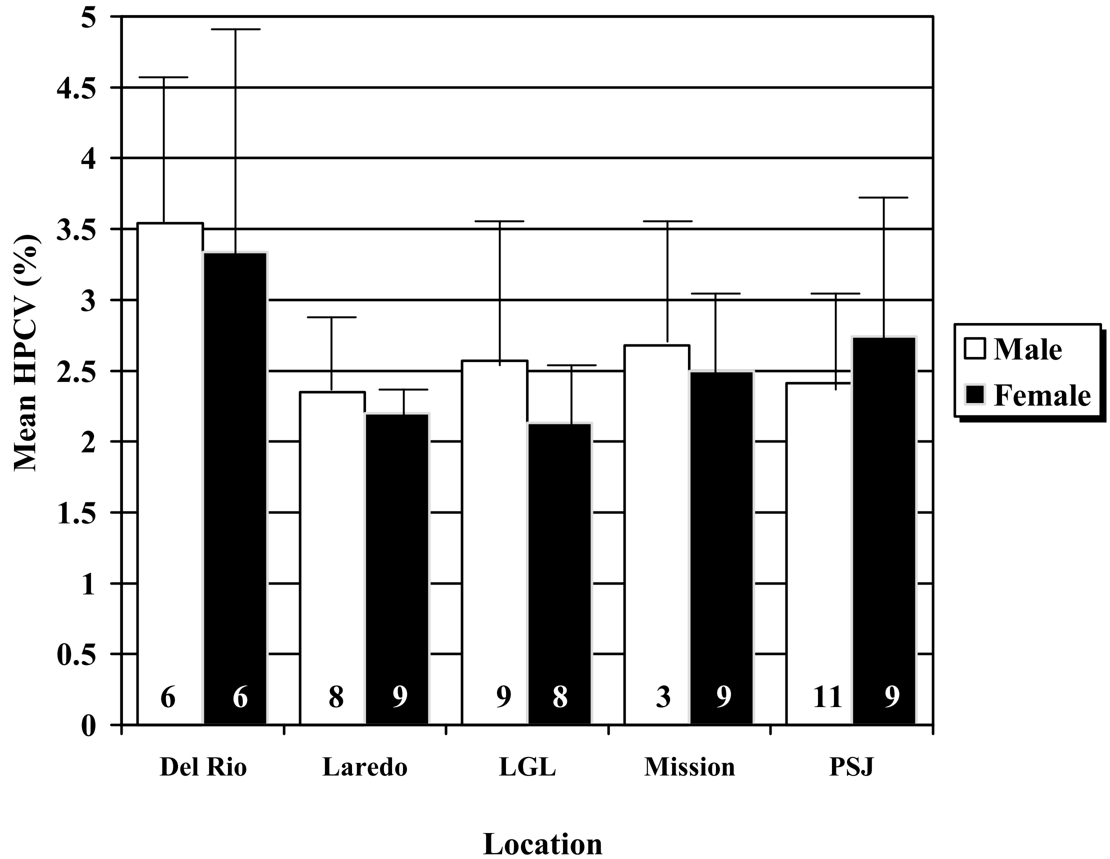

Figure 11. A gender comparison of half peak coefficient of variation in DNA content from blood cells taken from cave swallows (*Petrochelidon fulva*) collected from various sites along the Rio Grande during 1999. Bars represent standard deviations. Numbers in bars represent sample size.

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female cave swallows were pooled and used to analyze differences between locations since there were no sex-specific differences. Only cave swallow samples from Llano Grande Lake and Pharr-San Juan were collected during both field seasons; therefore, only these sites were used for seasonal comparisons. HPCV in DNA content was significantly higher (Figure 12, $P < 0.001$) in cave swallows from Llano Grande Lake in 2000 than in 1999. There were no significant differences (Figure 12, $P > 0.05$) in HPCV in cave swallows from Pharr-San Juan when data were compared between seasons. HPCV geometric mean was significantly higher (Figure 12, $P < 0.005$) in cave swallows from Del Rio than in cave swallows from other sites during 1999. During the 2000 field season, geometric means from Llano Grande Lake were significantly higher (Figure 12, $P < 0.001$) than HPCV geometric means from El Paso and Pharr-San Juan.

DNA variations were similar among male and female ($P > 0.05$) cliff swallows sampled in 1999 from Falcon Lake. Gender comparisons were not possible with other sites due to small sample sizes. Among cliff swallows captured in 1999, mean HPCV of DNA was significantly higher (Figure 13, $P < 0.001$) in birds from El Paso than in those from Falcon Lake and Somerville, but was similar to those from Brownsville. Genetic variation in cliff swallows from Brownsville was significantly higher (Figure 13, $P < 0.001$) than those from Somerville, but not from Falcon Lake or El Paso. Comparisons for mean HPCV of DNA among cliff swallow colonies sampled in 2000 were not feasible due to small sample sizes in several locations.

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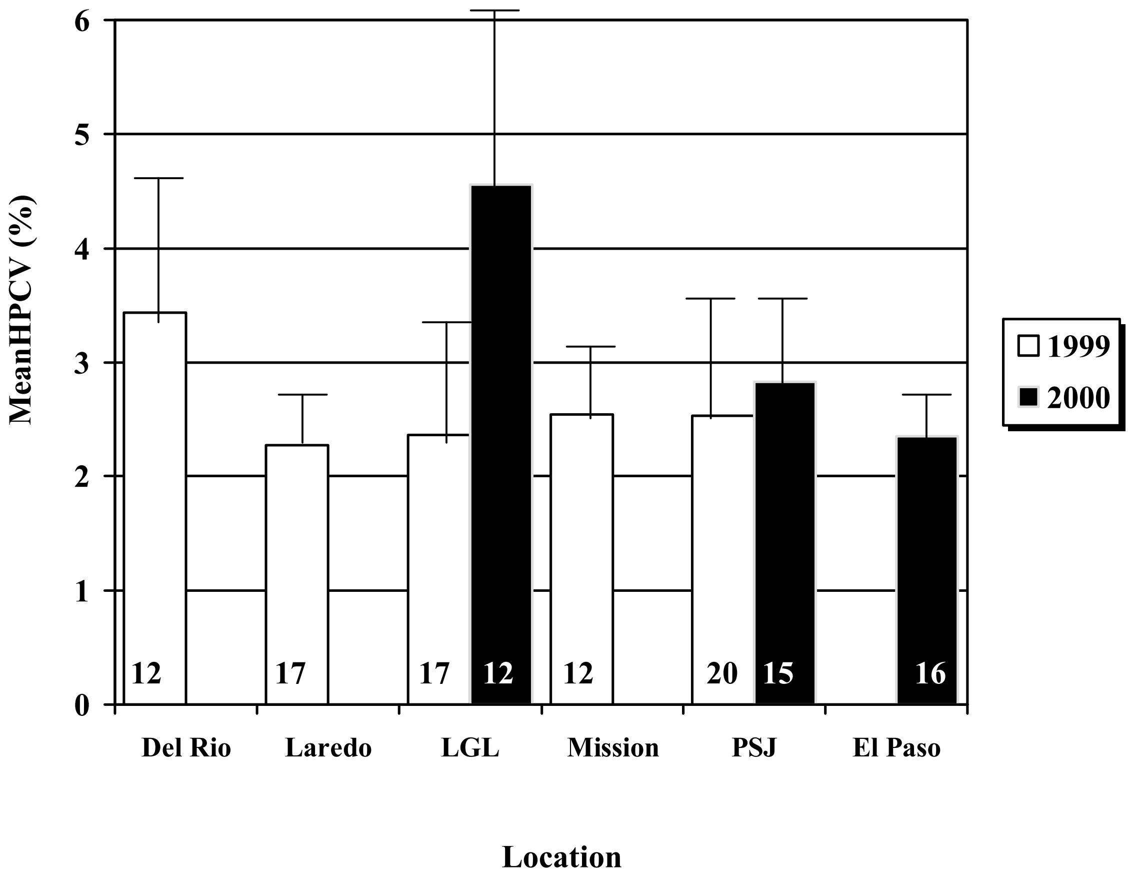

Figure 12. Half peak coefficient of variation in DNA content from blood cells taken from cave swallows (*Petrochelidon fulva*) collected from various sites along the Rio Grande during 1999 and 2000. Bars represent standard deviations. Numbers in bars represent sample size.

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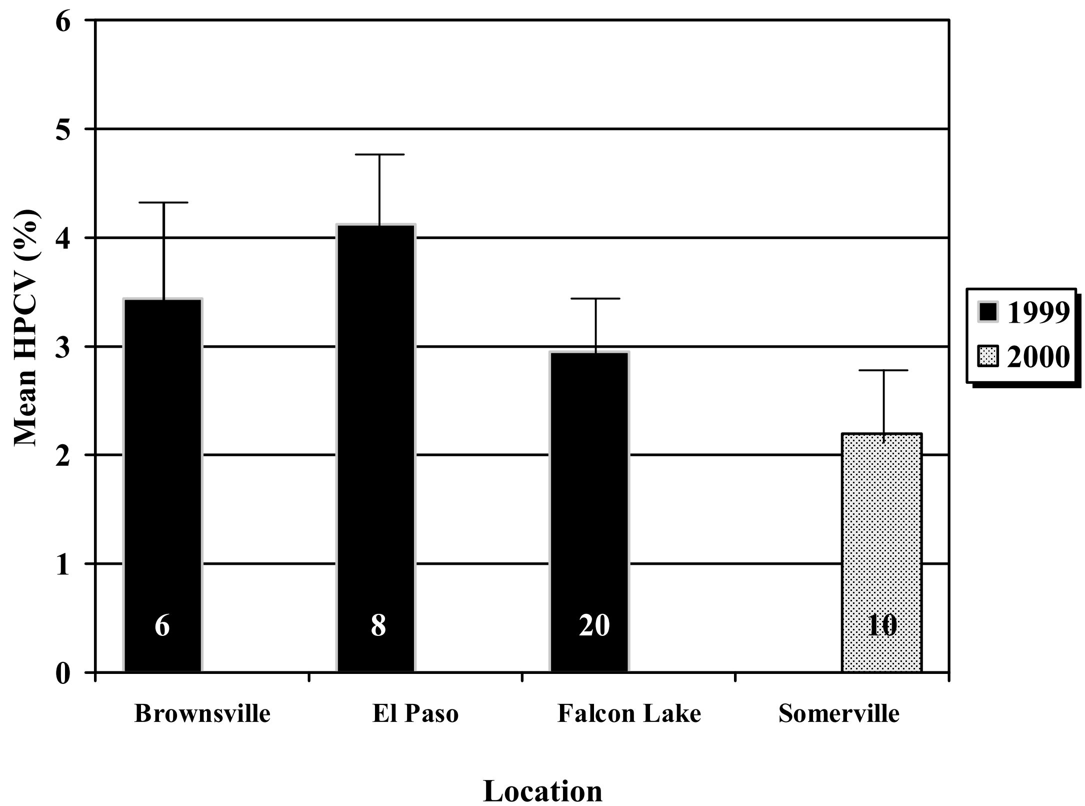

Figure 13. Means for half peak coefficient of variation in DNA content from blood cells taken from cliff swallows (*Petrochelidon pyrrhonota*) collected from various sites along the Rio Grande during 1999 and 2000. Bars represent standard deviations. Numbers in bars represent sample size.

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Thyroid Hormones

Validation of Radioimmunoassay for Cave Swallows

Minimum detectable limits for T_4 in this radioimmunoassay was as 0.2 ng/ml. Range tests results showed there were very low circulating levels of T_3 in cave swallows (0 to 4 ng/ml), with a mean of 0.74 ng/ml. For T_4, levels ranged from approximately 2 to 38 ng/ml and had a mean of 12.5 ng/ml. The spike recovery test showed that 104.5% recovery of native T_4 that was introduced into wild samples (Figure 14). T_3 samples were below minimum limits for the assay, therefore, recovery for this hormone was not tested. Serial dilutions of pools of wild samples of cave swallow plasma were parallel to each other, but the serially diluted T_4 standard did not represent the entire linear range of the standard curve (Figure 15). A parallelism test was not conducted for T_3. Variability within and between individual assays conducted in this study was measured though intraassay and interassay tests. The intraassay variability (CV = 10.27; n = 7) and interassay variability (CV = 8.84; n = 5) for T_4 were low, which indicate that protocols used to measure thyroid hormones in cave swallows were valid.

Circulating Thyroid Hormone Levels

No significant differences (Figure 16 and 17, $P > 0.05$, in all cases) in T_4 were detected between male and female cave swallows from each location during both years. Among cave swallows sampled in 1999, T_4 (1.7 to 38.79 ng/ml) did not differ significantly (Figure 18). Thyroxine levels in cave swallows collected during 2000 (10.1 to 12.6 ng/ml) did not differ significantly between sites (Figure 19, $P > 0.05$).

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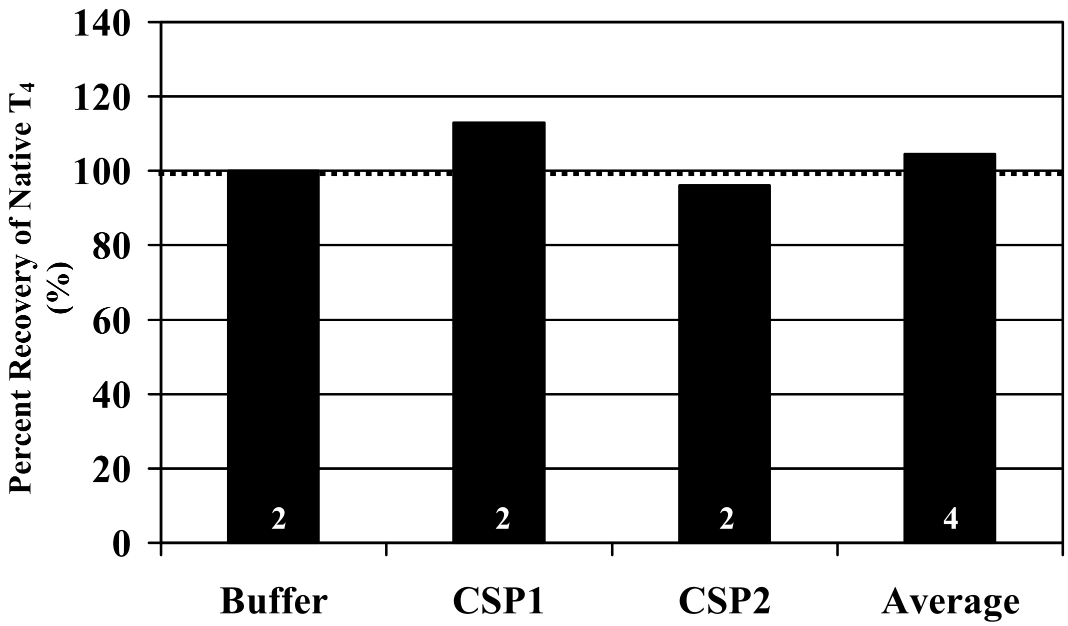

Figure 14. T_4 recovery test results. RIA buffer, cave swallow plasma pool 1 (CSP1), and cave swallow plasma pool 2 (CSP2), were spiked with native T_4. The cave swallow pool average recovery was 104.5%. Numbers in bars represent sample size.

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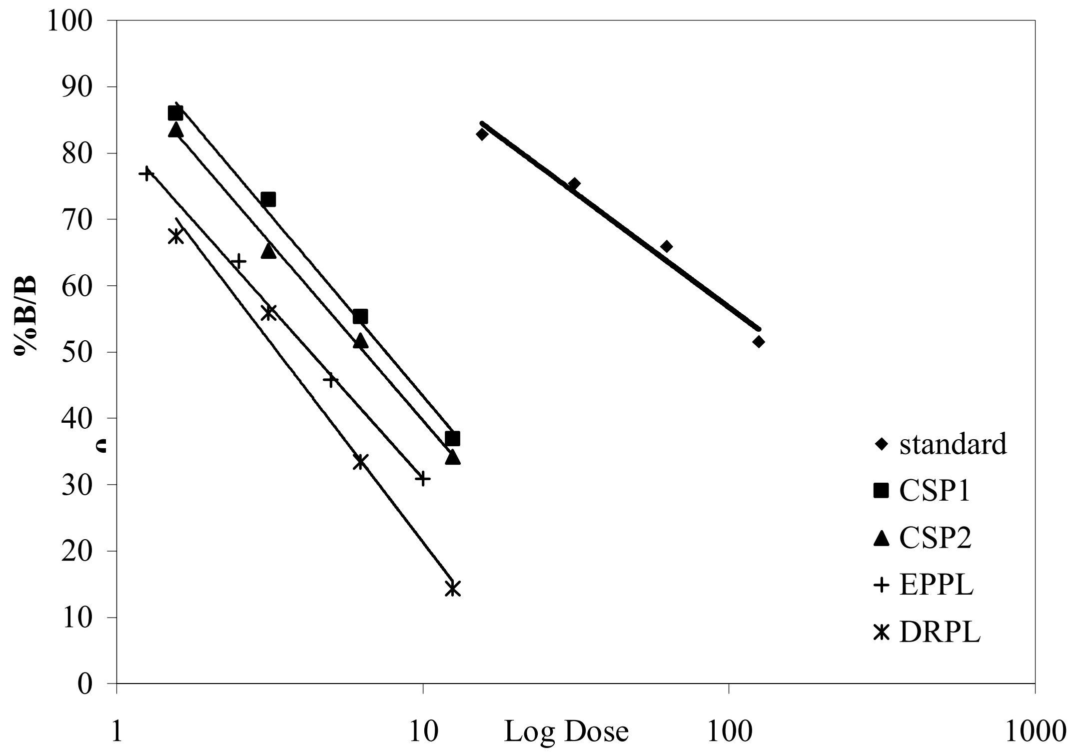

Figure 15. Results from the L-thyroxine parallelism test. Numbers represent pooled plasma from El Paso (EPPL), Del Rio (DRPL), and all sites (CAPO). Lines represent a best fit of the corresponding data. Cave swallow pools are in µl, and standard curve is in picograms/tube.

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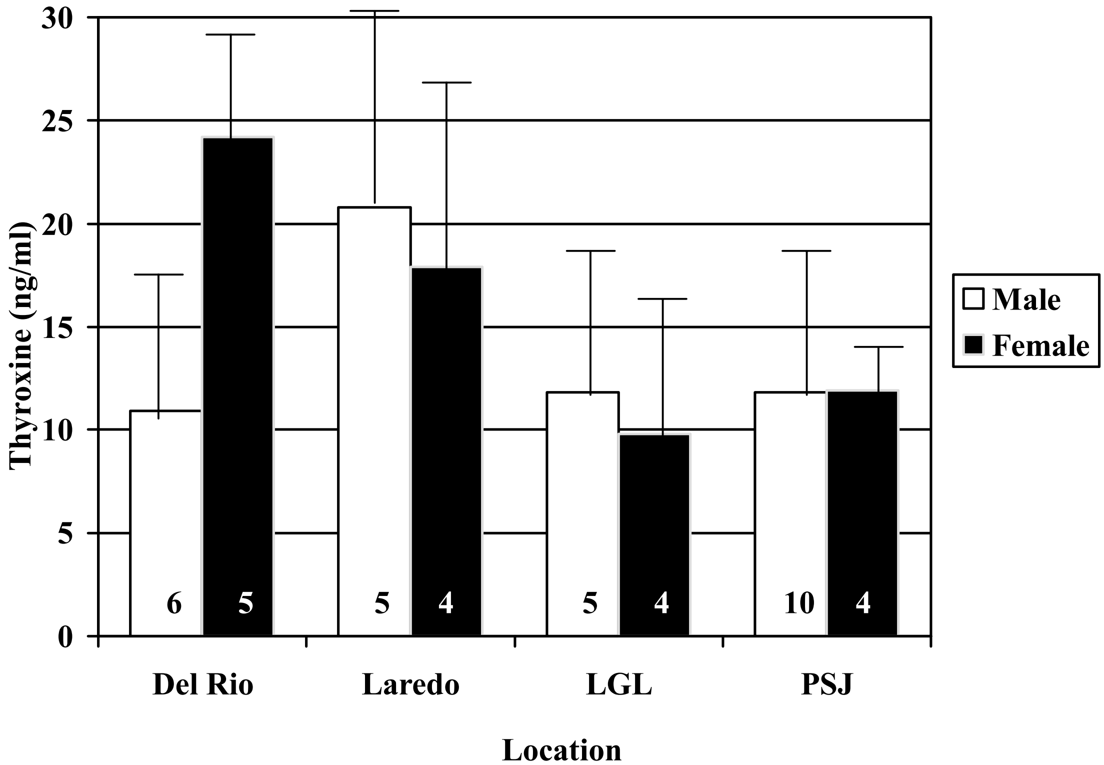

Figure 16. Circulating levels of thyroxine (T_4) in plasma samples from cave swallows (*Petrochelidon fulva*) collected from various sites along the Texas segment of the Rio Grande during 1999 (male vs. female). Bars represent standard deviations. Numbers in bars represent sample size.

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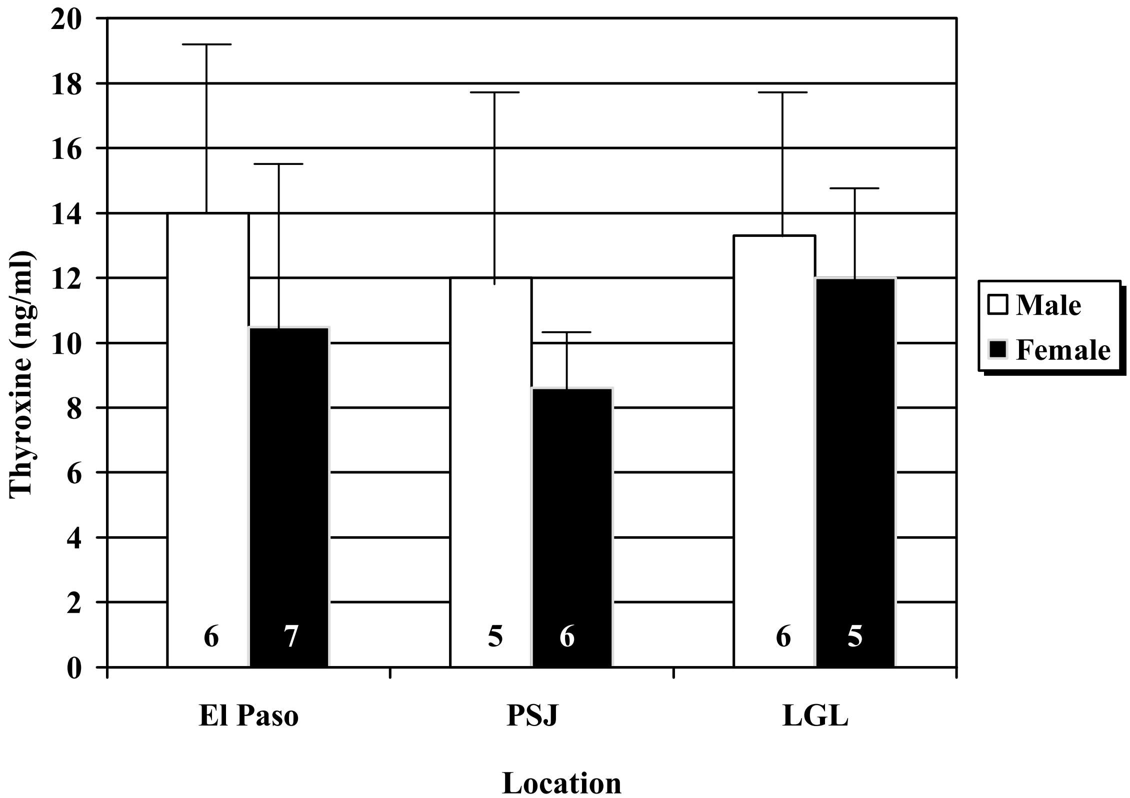

Figure 17. Circulating levels of thyroxine (T_4) in plasma samples from cave swallows (*Petrochelidon fulva*) collected from various sites along the Texas segment of the Rio Grande during 2000 (male vs. female). Bars represent standard deviations. Numbers in bars represent sample size.

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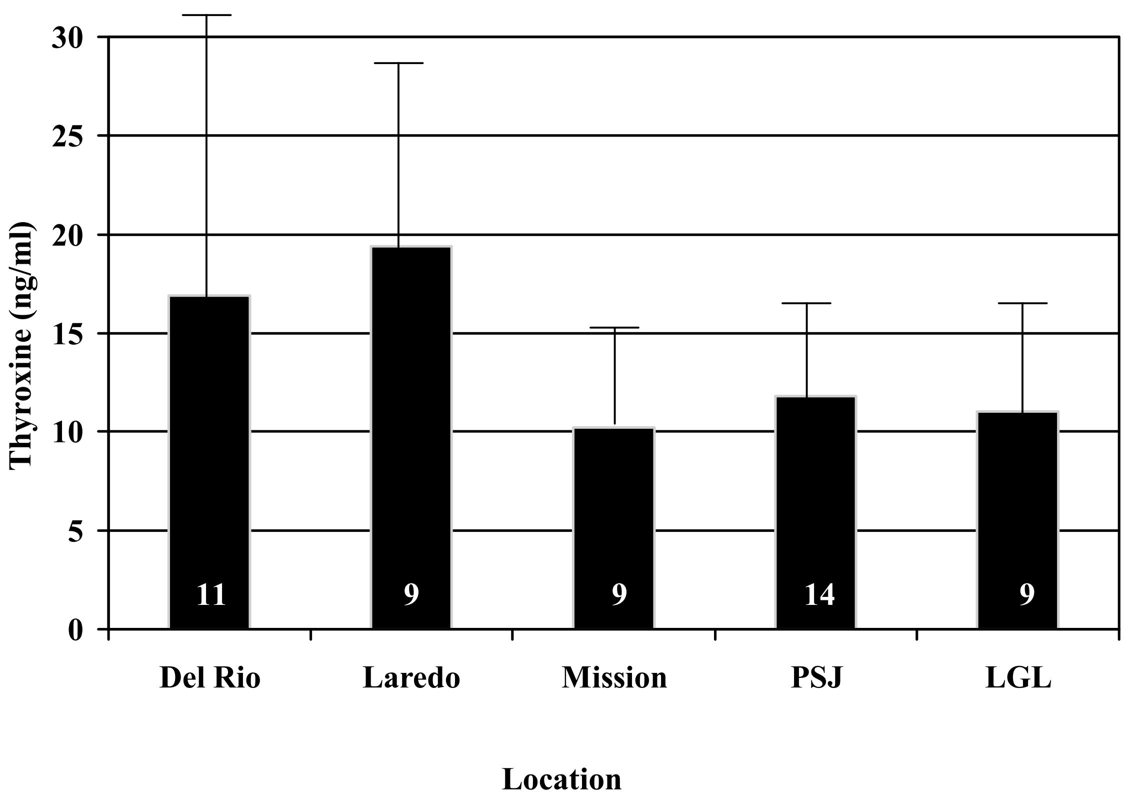

Figure 18. Circulating levels of thyroxine (T_4) in plasma samples from cave swallows (*Petrochelidon fulva*) collected from various sites along the Texas segment of the Rio Grande during 1999. Bars represent standard deviations. Numbers in bars represent sample size.

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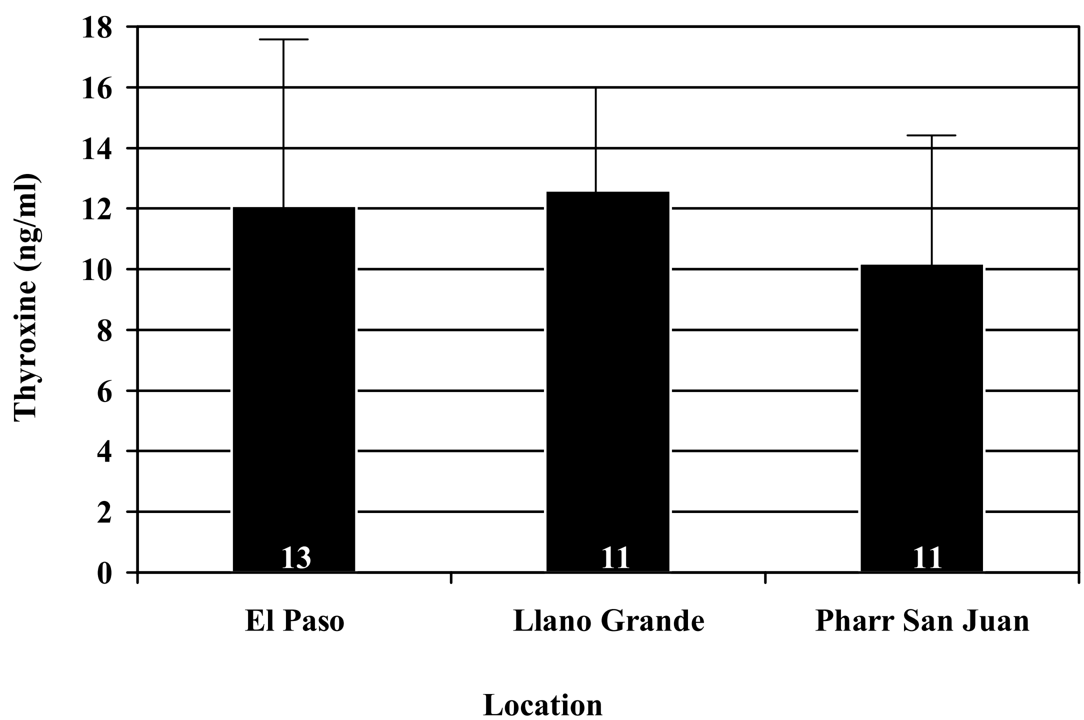

Figure 19. Circulating levels of thyroxine (T_4) in plasma samples from cave swallows (*Petrochelidon fulva*) collected from various sites along the Texas segment of the Rio Grande during 2000. Bars represent standard deviations. Numbers in bars represent sample size.

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DISCUSSION

Organochlorines

To this date, no studies on pesticide or contaminant residues have been conducted on cave swallows (West 1995). Because of the small sample size, data acquired for cave swallow samples only exhibit the presence or exposure of certain classes of chemicals to these birds, but do not indicate levels potentially found in the population. Biomarkers may identify responses associated with contaminant exposure, but establishment of cause and effect relationships was not possible. DDT and DDE levels in birds from this study were lower than concentrations known to cause abnormalities in birds (Blus 1996). DDE is generally found in greater concentrations than its persistent parent compound (Stickel et al. 1984; Bishop et al. 1995) and is the principal metabolite associated with eggshell thinning (Blus 1996). A review by Mora and Wainwright (1997) identified high levels of DDE for western kingbirds (*Tyrannus verticalis*) (60.9 µg/g ww) collected in 1982 from Hudspeth County (Hunt et al. 1986), in laughing gulls (71 µg/g ww) from Llano Grande Lake in 1978 (White et al. 1983), and in white pelican carcasses (46 µg/g ww) from the Pharr Settling Basin (Gamble et al. 1988). Mora and Wainwright (1997) indicated that DDE residues above 5 µg/g had not been reported in birds since 1986, since it had peaked in the early 1980s; however, green heron (*Butorides virescens*) eggs sampled in 1997 contained DDE levels as high as 10,954 ng/g ww (Wainwright et al. 2001). Insectivorous birds accumulate fewer concentrations of persistent organic chemicals than piscivorous birds since they are

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lower in the food chain. This may partly explain why DDE residue levels in cave and cliff swallows from Llano Grande Lake and El Paso were not as high as black-crowned night heron eggs collected in 1996 by Wainwright et al. (2001). For comparative purposes, whole body DDE levels in a tree swallow study from the Great Lakes ranged from 0.0272-1.484 ug/g; furthermore, reproduction of tree swallows appeared to be unaffected by organochlorine contamination at these levels (Bishop et al. 1999).

Total PCB concentrations in tissue samples obtained from cave swallows from Llano Grande Lake, and cliff swallows from El Paso were lower than levels known to cause reduced hatching, embryo mortality, and deformities in birds (Hoffman et al. 1996). For comparison, total PCB concentrations in tree swallows sampled from the Great Lakes in a recent study ranged from 0.038-5.469 ug/g, and did not appear to affect reproduction (Bishop et al. 1999). PCB levels were once as high as 4,000 ng/g ww (White et al. 1983) in laughing gulls from Llano Grande Lake, which were the highest levels that have been recorded for LRGV birds (Mora and Wainwright 1997). However, recent studies conducted on the Rio Grande ecosystem indicate downward trends in PCB concentrations from environmental media, and are not as prevalent as they were two decades ago (Mora 1996a; Garcia et al. 2001; Wainwright et al. 2001).

HCH isomers, including lindane and all other OCs, were found at low levels in birds examined in this study. None of the cyclodiene pesticides recommended by the Texas Agricultural Extension Service for controlling cotton pests in the Lower Rio Grande Valley (Norman and Sparks 2001; table 1) were detected in carcass samples obtained in this study.

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Trace Metals

For trace metals, mercury, lead, and arsenic were below detection in the samples analyzed in this study. A recent assessment by Mora et al. (2001) indicated that mercury and selenium concentrations were low and not of concern in fish that reside in resacas (oxbow lakes) along the lower Rio Grande. Schmitt and Brumbaugh (1990) found high As levels in fish collected from the Brownsville area during the mid 1980s, but more recently, high levels of As were found in fish from a Resaca located in Matamoros, Mexico (Mora et al. 2001), an area located a few kilometers from the cave and cliff swallow colonies near Brownsville. Not much is known about previous mercury or lead contamination in areas near cave and cliff swallow colonies. Selenium concentrations were detected at low levels in cave and cliff swallow carcasses. Sublethal effects are considered likely when liver concentrations exceed 10 ppm ww (Heinz 1996). Selenium concentrations in cliff swallow eggs from a reference site used in a Kesterson Reservoir selenium study in 1984 ranged from 1.0-1.4 µg/g dw (Heinz 1996). In this study, the highest selenium concentration (0.79 ppm) was considerably lower than levels known to cause harm in birds (Heinz 1996). Cadmium was not detected in cave swallows from Llano Grande Lake, and birds from El Paso had considerably low levels. Cadmium levels have ranged from 0.07 to 3.3 µg/g dw in birds from the Rio Grande (Mora and Wainwright 1997).

Excessive nickel concentrations may have a potential role in wildlife genotoxicity. Nickel may cause genetic damage because it is able to bind to DNA and proteins in cells *in vitro* and to chromatin *in vivo* (Herkovits et al. 1999). Nickel-induced abnormal DNA repair may be a mechanism for carcinogenesis (Au et al. 1994; Hartman and Hartwig 1998). Few studies have reported nickel concentrations in birds inhabiting the Rio Grande. TNRCC (1994) reported nickel in sediments and fish sampled along the Rio Grande; fish tissue concentrations ranged from 0.04 mg/kg ww in carp from the Rio Conchos/Rio Grande confluence to 6.93 mg/kg ww in channel catfish from an El Paso site, a site that was less than 1 km away from the sampling site in El Paso.

Chromium, a known genotoxic, was detected at lower levels (0.47 to 2.39 ug/g dw) than those reported in previous Rio Grande studies (Mora and Wainwright 1997). Results from a binational study conducted by the TNRCC (1994) indicate that chromium was detected in 100% of sediment samples and 57.6% in tissue samples (45 sites) taken from sites along the Rio Grande (Mora and Wainwright 1997). Chromium-induced DNA damage may be produced through a variety of mechanisms. The reduction of Cr (VI) to Cr (V) and Cr (IV) to Cr (III) may generate reactive oxygen species that induce oxidative damage to cellular molecules, including DNA (Casadevall et al. 1999; Flores and Perez 1999; Blasiak and Kowalik 2000). Other studies suggest that chromium may exert its mutagenic effects through the production of either crosslinks or intrastrand crosslinks with DNA (Kohn 1983; Herbert and Luiker 1996). Sugden et al. (2001) suggest that direct oxidation of guanine and 7,8-dihydro-8-oxoguanine in DNA by a high-valent chromium complex may be a possible mechanism for chromate genotoxicity. Based on the ubiquity of chromium in the Rio Grande ecosystem, genetic damage due to chromium exposure is a significant concern for the health of insectivorous birds.

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Flow Cytometric Analyses

There were no sex-related differences in DNA variation in cliff and cave swallows sampled in this study. Reports have shown an absence of sex-related differences in contaminant-induced chromosome damage in various mammals (Bender et al. 1988; Heddle 1990; Gauthier et al. 1999). The lack of sex-related differences in DNA variation indicates that both male and female swallows are equally susceptible to clastogenic events.

Cave swallows from Del Rio had the highest HPCV along with the highest spleen mass among all locations. The spleen is one of the major organs of the immune system (Blanco et al. 2001). Some studies suggest that increased spleen weights may result from acute exposure to methoxyclor (Ahmed 2000), chromium (Glaser et al. 1985), and parasites (Blanco et al. 2001). Prolonged spleen enlargement is a condition known as spleenomegaly, and is caused by increased lymphocyte production (John 1994). It is known that certain contaminants or pathogens may increase lymphocyte production as an immune response; however, splenic atrophy due to lymphocyte depletion may indicate prolonged contaminant exposure or acute immunosuppressive dosages. Contaminant data for wildlife of Val Verde County is scarce; however, rural land use surrounding the Del Rio area is remarkably dissimilar to other sampling locations from the LRGV and El Paso (personal observations). Goat and sheep ranching are the primary economy for the Del Rio region; therefore, large inputs of agricultural pesticides are not expected. High levels of trace metals in sediments, especially chromium, have been problematic in the Del Rio area. A binational study by the

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TNRCC (1994) found that sediments sampled from the Arroyo de las Vacas, a tributary emptying into the Rio Grande from the state of Coahuila, Mexico, near Del Rio had the second highest level of chromium (43.9 mg/kg dw) in the Rio Grande segment from El Paso to Brownsville. Flow cytometry results for cave swallows from Del Rio indicate possible exposure to clastogenic chemicals. Increased spleen and liver weight are characteristic of immune response and enzyme-induction (e.g. ethoxyresorufin-O-dealkylase and p450-associated monooxygenase), and may suggest PAH exposure. Based on biomarker response and known elevated chromium levels in the region, it appears that genetic damage could have occurred through chromium exposure, although non-point sources of PAHs could also be likely.

Cave swallows collected in 2000 from Llano Grande Lake showed signs of recent contamination since the HPCVs in swallows collected in 1999 were significantly lower. Cave swallows from the Pharr-San Juan site near Llano Grande did not show differences in DNA variation between years, which suggest that something different may be occurring in Llano Grande Lake, an area previously known as a contaminant hot spot. Previous studies have shown that PAHs can cause chromosomal breakage (Matsuoka et al. 1982; Djomo et al. 1995). McBee and Bickham (1988) reported broader range of CVs in DNA content and chromosome aberrations in mice collected at petrochemical-contaminated sites than at control sites. Higher DNA CVs were also reported in blood collected from black-crowned night herons at a petroleum-contaminated Louisiana site (Custer et al. 1994) compared to other sites in that study. In previous years, Llano Grande Lake has been reported for having high DDE, PCB, toxaphene, and endosulfan

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levels (Mora and Wainwright 1997); however, these compounds are not known to cause genetic damage. PCBs, however, were shown to cause DNA oxidation in a recent report (Hassoun et al. 2001); although, these findings were limited to hepatic and brain tissues. Moreover, a lack of significant relationships between genetic damage in blood cells and total PCBs or toxic equivalents were reported in lesser scaups (*Aythya affinis*) wintering in a heavily polluted Indiana Harbor Canal (Custer et al. 2000). It is possible that cave swallows from Llano Grande Lake may have been exposed to PAH residues from urban sources. Urban creeks and drainages from Donna, Weslaco, and Mercedes all drain into Llano Grande Lake. Furthermore, FM 1015 is a major artery with increased truck and automobile traffic that stems from the international bridge from Nuevo Progreso, Mexico and Progreso, Texas. This thoroughfare may significantly add to PAH deposition. Collectively, aquatic contamination by PAHs is caused by petroleum spills, discharges, and seepages; industrial and municipal wastewater; urban and suburban run-off; and atmospheric deposition (Albers 1995).

Cave and cliff swallows were collected at the Resaca de Rancho Viejo, a segment of the Rancho Viejo Floodway, which also receives agricultural inputs. This site is also 0.5 miles away from the Brownsville Ship Channel (BSC), an area of concern for exposure to contaminants in biota. Davis (1995) also reported trace metal concentrations of chromium (0.275 μg/g ww) and copper (0.556 μg/g ww) in spot croakers (*Leistomus xanthurus*) from San Martin Lake, an area adjacent to the BSC. The highest concentrations of PAHs were detected in oysters (3.38 μg/g ww; Jackson et al. 1994) sampled in 1984 and 1988 from South Bay, which is also adjacent to the BSC.

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Increased genetic variation may have resulted from chronic petrochemical exposure, as demonstrated by Davis (1995), or could have resulted from a combination of petrochemical and trace metal exposure.

Cliff swallows collected in 1999 from El Paso had the highest DNA variation than all cliff swallows from the study, and were also significantly higher than cliff swallows from Falcon Lake and the Somerville reference site. The wastewater facility adjacent to the El Paso study site empties part or all of its discharge into Franklin Canal (personal observations), the main water source for these swallows. Canals associated with the El Paso wastewater treatment facility have been recorded as having the highest concentrations of certain trace metals in sediment (Mora and Wainwright 1997); for example Hg (1.51 µg/g dw), Se (4.5 µg/g dw), Pb (81 µg/g dw), Cr (45 µg/g dw), Cd (2.4 µg/g dw), Zn (392 µg/g dw) were the maximum concentrations found in the binational study (TNRCC 1994). It is clear that swallows nesting along these canals near El Paso are exposed to significant concentrations of trace metals. Of the trace metals reported in the binational study (TNRCC 1994), Cr is the only one with genotoxic capacities. While chromium has been found in high concentrations in sediments near the El Paso sampling site and is potentially the primary clastogenic source, PAH exposure cannot be ruled out. The El Paso site was revisited during June 2000, however, mostly cave swallows were collected on the second visit. Since cliff swallows are known for their high nest fidelity between breeding seasons (Brown and Brown 1995), it is highly unlikely that these birds selected a different nesting site during the 2000 breeding season. Moreover, it may be possible that unhealthy birds from this colony did not

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survive after 1999, or cave swallows may have gained a nest-selecting competitive advantage since they have recently been found to over winter in Texas (Lasley and Sexton 1991; West 1995).

The Somerville, TX, reference site had the lowest DNA variation of all birds sampled during both field seasons. This finding suggests that birds nesting along the Rio Grande may have higher exposure to clastogens than birds from Somerville. The Somerville sampling site is located approximately 300 miles north of the nearest sampling site along the Rio Grande. Potential contaminant exposure to these birds is mainly through agrochemicals associated with cotton farming. Based on flow cytometry results, it does not appear that cliff swallows collected from Somerville were highly exposed to chemical clastogens.

Thyroid Hormones

Conclusive results for thyroid hormones obtained by this study are limited due to several factors. Sample sizes, especially for cliff swallows, were generally small. Obtaining plasma from cave and cliff swallows was relatively simple; however, in El Paso and Brownsville, samples sizes were reduced due to hemolysis. For 1999, 13 samples (22.8%) were removed due to hemolysis, while none were hemolyzed in 2000. Hemolysis, based on field observations, was a result of blood exposure to air and local tissue damage that occurred at the sampling site, which occurred when jugular veins were difficult to isolate due to their small size.

The RIA procedures from Leiner et al. (2000) were then validated for cave swallow plasma samples. The range test revealed that T_3 was present at levels below detection by this assay. T_4 was present at considerably higher levels than T_3, and corresponded with thyroid hormone levels identified in other passerines, including tree swallows (Bishop et al. 1998).

Thyroid hormones undergo diurnal cycles that fluctuate according to dynamic environmental conditions, such as photoperiod (Dawson et al. 2001; Bernard et al. 1997). In birds, thyroid hormones play a role in the transmission of photoperiod signals to the suprachiasmatic nucleus (Dawson et al. 2001), and in tree swallows, Reinert and Wilson (1996) indicate that increasing thyroxine concentration in plasma, after photostimulation, serves to program the gonadal cycle and molt. In birds, thyroid function can affect reproductive function; therefore, birds from this study were sampled at approximately the same time at each site to reduce these factors. As reported earlier, there were no significant differences in gonad mass between locations among males, and females as well. Results from this study serve as a means of detecting gender-specific differences in hormone levels between members of the same colony, and as an indicator of thyroid activity at the time of collection. Results, however, indicate that hormone levels did not differ significantly between locations, and may have been a result of our sampling scheme. Advances in photoneuroendocrinology have linked thyroid hormones to the process of seasonal reproduction in various bird species (Reinert and Wilson 1996). In this study, cave and cliff swallows did not show any sex-related differences in T_4 during both sampling years. Experiments by Wilson and Reinert (1993, 1995) revealed that exogenous L-thyroxine induces testicular growth in photosensitive American tree sparrows (*Spizella arborea*) retained on short days (8hr light: 16 hr dark)

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and further augments testicular growth when birds are moved to days with increased photoperiods. In female American tree sparrows, circulating T_4 increases early during photostimulation while initiating increasing levels of GnRH-I, pituitary LH, plasma LH, and ultimately ovarian mass (Reinart and Wilson 1996). Results from this study support evidence that thyroid hormones are equally important in male and female birds for proper reproductive function.

Low levels of 3,5,3'-triiodothyronine in plasma sample of cave and cliff swallows did not come as a surprise. In chicken serum, T_3 is normally one-tenth the concentration of T_4 (Newcomer 1974; Chang et al. 1999). If this were the case in cave and cliff swallows, the projected levels of T_3 based on circulating T_4 would be in the range of approximately 0.2 to 3.8 ng/ml, which agrees with findings in other passerine studies. For example, Bishop et al. (1998) reported 1.54 ng/ml T3 in male tree swallow chicks from a nonsprayed control site. Singh et al. (1997) reported 6.5 ng/ml in adult male blue rock pigeons (*Columbia livia intermedia*) used as controls, although these birds also weighed ten times more that the average tree and cave swallow.

Cave swallows from this study had similar T_4 levels. Possible explanations for variability in thyroid levels in birds from these sites include: diet quality and food restriction (Bruggeman et al. 1997), reproductive status (Dawson et al. 2001), temperature (Decauypere and Kühn 1988), and PCB exposure (Janz and Bellward 1997). Thyroxine concentrations are known to increase in food-restricted domestic fowl (Bruggeman et al. 1997); however, based on overall body mass and the presence of stomach contents, food deprivation was probably not a factor in these birds.

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Thyroid hormone levels reported in this study are the first to be documented for these birds. Further sampling and complete tissue-contaminant analysis, would help establish whether these levels were indicative of hypo or hyperthyroidism. The presence of absence of thyroid hormone disruption in cliff and cave swallows in this study could not be concluded. For comparative purposes, T_4 levels found in these birds were in accordance with levels (10 ng/ml) regularly seen in laying hens (Decuypere and Kühn 1988). In another study, non-breeding, captive, great blue herons recorded mean plasma T_4 levels of 39 ng/ml (Janz and Bellward 1997).

Overall, the data gathered for thyroid hormones was limited in this study. For a more comprehensive approach, continuous seasonal sampling is recommended to measure seasonal fluctuations in thyroid hormones. A study design that incorporates seasonal sampling to determine pre-migratory (spring), pre-ovulatory, ovulatory, egg-laying, post egg-laying, and pre-migratory (fall) thyroid hormone levels would probably provide more insight into seasonal thyroid hormone dynamics. Taking approximately 6 blood samples per season, per location, would translate to an unacceptably large number of birds that would eventually have to be sacrificed (due to a relatively large volume of blood required for plasma extraction and RIA analysis); therefore, this study was limited to a once per season, sampling scheme. Histological data would further support evidence for disruption of thyroid function, and dose-response studies would further elucidate cause and effect relationships between organochlorines and thyroid function. In the future, complete contaminant screening would support evidence for the detection of exposure and effects of endocrine disrupting chemicals.

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CONCLUSIONS

This study is the first to assess the efficacy of cliff and cave swallows as indicators of contamination along the Rio Grande ecosystem using flow cytometry, and thyroid radioimmunoassays. An advantage for using thyroid hormone and genetic biomarkers is that temporal patterns of hormone levels and trends in genetic variation for individual colonies may be compared yearly, and long-term trends may therefore be established and evaluated.

Flow cytometry was useful in detecting geographic differences in DNA variation. Specifically, the data showed that cave swallows located in Del Rio may possibly be exposed to environmental clastogens since their coefficient of variation in DNA content was significantly higher in these birds than in cave swallows sampled from other colonies along the Rio Grande. Evaluations of specific contaminant loads were not feasible in these birds; however, data in the literature indicate that high levels of chromium have been recorded in tributaries near Del Rio in the mid 1990s. Exposure to PAH contamination may also be possible. Increased DNA variation was also noted in Llano Grande Lake cave swallows from 1999 to 2000. This indicates that high levels of genotoxins may have been present in cave swallows during 1999, and detected one year later in DNA content.

Thyroid hormone radioimmunoassays protocols used by Leiner et al. (2000) require additional modifications to accurately detect circulating levels of T_3 in cave

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swallows. Levels of 3,5,3'-triiodothyronine were below the minimum levels of detection for this assay.

There was no evidence of hypo or hyperthyroidism based on the data presented in this study. Direct linkage to thyroid hormone disruption and organochlorines requires complete contaminant screening and yearlong sampling to incorporate seasonal variations in hormone concentrations. There were no gender related differences in cave swallows sampled in this study. This may have been attributed to the dependence of thyroid hormones and photoperiod on seasonality (Dawson et al. 2001; Wilson and Reinert 1993, 1995).

Findings from this study suggest that cliff and cave swallows are excellent biomarkers for DNA-damaging contaminants. Only 4-5 drops of blood are necessary for flow cytometry; therefore, non-invasive procedures, such as nail clipping, to obtain blood drops, eliminates the need to sacrifice a large number of birds in the future. Although cliff and cave swallows are known for their nest fidelity between years (Brown and Brown 1995, West 1995, Sikes and Arnold 1984), bird banding, coupled with yearly sampling, could provide more information on the population dynamics of these birds. Based on genetic biomarker data gathered from this study, areas of concern for environmental contaminants include the areas near Llano Grande Lake, Del Rio, and El Paso.

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